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(54) **VON WILLEBRAND FACTOR VARIANTS HAVING IMPROVED FACTOR VIII BINDING AFFINITY**

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(57) **ABSTRACT**

The present invention relates to a polypeptide comprising a modified von Willebrand Factor (VWF) having a higher Factor VIII binding affinity than non-modified VWF, its pharmaceutical use and method of its preparation.

20 Claims, No Drawings

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VON WILLEBRAND FACTOR VARIANTS HAVING IMPROVED FACTOR VIII BINDING AFFINITY

FIELD OF THE INVENTION

The present invention relates to a polypeptide comprising a modified von Willebrand Factor which exhibits improved binding affinity to Factor VIII. The invention further relates to a complex comprising the polypeptide and FVIII, to a polynucleotide encoding the polypeptide of the invention and a method of producing the polypeptide. Furthermore, the invention concerns the therapeutic or prophylactic use of the polypeptide or complex of the invention for treating bleeding disorders.

BACKGROUND OF THE INVENTION

There are various bleeding disorders caused by deficiencies of blood coagulation factors. The most common disorders are hemophilia A and B, resulting from deficiencies of blood coagulation factor VIII and IX, respectively. Another known bleeding disorder is von Willebrand's disease.

In plasma FVIII exists mostly as a noncovalent complex with VWF and its coagulant function is to accelerate factor IXa dependent conversion of factor X to Xa. Due to the complex formation of FVIII and VWF it was assumed for a long time that FVIII and VWF functions are two functions of the same molecule. Only in the seventies it became clear that FVIII and VWF are separate molecules that form a complex under physiologic conditions. In the eighties then the dissociation constant of about 0.2 nmol/L was determined (Leyte et al., Biochem J 1989, 257: 679-683) and the DNA sequence of both molecules was studied.

Classic hemophilia or hemophilia A is an inherited bleeding disorder. It results from a chromosome X-linked deficiency of blood coagulation FVIII, and affects almost exclusively males with an incidence of between one and two individuals per 10.000. The X-chromosome defect is transmitted by female carriers who are not themselves hemophiliacs. The clinical manifestation of hemophilia A is an increased bleeding tendency. Before treatment with FVIII concentrates was introduced the mean life span for a person with severe hemophilia was less than 20 years. The use of concentrates of FVIII from plasma has considerably improved the situation for the hemophilia A patients increasing the mean life span extensively, giving most of them the possibility to live a more or less normal life. However, there have been certain problems with the plasma derived concentrates and their use, the most serious of which have been the transmission of viruses. So far, viruses causing hepatitis B, non-A non-B hepatitis and AIDS have hit the population seriously. Since then different virus inactivation methods and new highly purified FVIII concentrates have recently been developed which established a very high safety standard also for plasma derived FVIII.

In severe hemophilia A patients undergoing prophylactic treatment FVIII has to be administered intravenously (i.v.) about 3 times per week due to the short plasma half-life of FVIII of about 12 to 14 hours. Each i.v. administration is cumbersome, associated with pain and entails the risk of an infection especially as this is mostly done at home by the patients themselves or by the parents of children being diagnosed for hemophilia A.

It would thus be highly desirable to create a FVIII with increased functional half-life allowing the manufacturing of pharmaceutical compositions containing FVIII, which have to be administered less frequently.

Several attempts have been made to prolong the half-life of non-activated FVIII either by reducing its interaction with cellular receptors (WO 03/093313A2, WO 02/060951A2), by covalently attaching polymers to FVIII (WO 94/15625, WO 97/11957 and U.S. Pat. No. 4,970,300), by encapsulation of FVIII (WO 99/55306), by introduction of novel metal binding sites (WO 97/03193), by covalently attaching the A2 domain to the A3 domain either by peptidic (WO 97/40145 and WO 03/087355) or disulfide linkage (WO 02/103024A2) or by covalently attaching the A1 domain to the A2 domain (WO2006/108590).

Another approach to enhance the functional half-life of FVIII or VWF is by PEGylation of FVIII (WO 2007/126808, WO 2006/053299, WO 2004/075923) or by PEGylation of VWF (WO 2006/071801) which pegylated VWF by having an increased half-life would indirectly also enhance the half-life of FVIII present in plasma. Also fusion proteins of FVIII have been described (WO 2004/101740, WO2008/077616 and WO 2009/156137).

VWF, which is missing, functionally defect or only available in reduced quantity in different forms of von Willebrand disease (VWD), is a multimeric adhesive glycoprotein present in the plasma of mammals, which has multiple physiological functions. During primary hemostasis VWF acts as a mediator between specific receptors on the platelet surface and components of the extracellular matrix such as collagen. Moreover, VWF serves as a carrier and stabilizing protein for procoagulant FVIII. VWF is synthesized in endothelial cells and megakaryocytes as a 2813 amino acid precursor molecule. The amino acid sequence and the cDNA sequence of wild-type VWF are disclosed in Collins et al. 1987, Proc Natl. Acad. Sci. USA 84:4393-4397. The precursor polypeptide, pre-pro-VWF, consists of a 22-residue signal peptide, a 741-residue pro-peptide and the 2050-residue polypeptide found in mature plasma VWF (Fischer et al., FEBS Lett. 351: 345-348, 1994). After cleavage of the signal peptide in the endoplasmatic reticulum a C-terminal disulfide bridge is formed between two monomers of VWF. During further transport through the secretory pathway 12 N-linked and 10 O-linked carbohydrate side chains are added. More important, VWF dimers are multimerized via N-terminal disulfide bridges and the propeptide of 741 amino acids length is cleaved off by the enzyme PACE/furin in the late Golgi apparatus. The propeptide as well as the high-molecular-weight multimers of VWF (VWF-HMWM) are stored in the Weibel-Pallade bodies of endothelial cells or in the α-Granules of platelets.

Once secreted into plasma the protease ADAMTS13 cleaves VWF within the A1 domain of VWF. Plasma VWF therefore consists of a whole range of multimers ranging from single dimers of 500 kDa to multimers consisting of up to more than 20 dimers of a molecular weight of over 10,000 kDa. The VWF-HMWM hereby having the strongest hemostatic activity, which can be measured in ristocetin cofactor activity (VWF:RCo). The higher the ratio of VWF:RCo/VWF antigen, the higher the relative amount of high molecular weight multimers.

Defects in VWF are causal to von Willebrand disease (VWD), which is characterized by a more or less pronounced bleeding phenotype. VWD type 3 is the most severe form in which VWF is completely missing, VWD type 1 relates to a quantitative loss of VWF and its phenotype can be very mild. VWD type 2 relates to qualitative defects of VWF and can be as severe as VWD type 3. VWD type 2 has many sub forms some of them being associated

with the loss or the decrease of high molecular weight multimers. Von VWD type 2a is characterized by a loss of both intermediate and large multimers. VWD type 2B is characterized by a loss of highest-molecular-weight multimers.

VWD is the most frequent inherited bleeding disorder in humans and can be treated by replacement therapy with concentrates containing VWF of plasmatic or recombinant origin. VWF can be prepared from human plasma as for example described in EP 05503991. EP 0784632 describes a method for isolating recombinant VWF.

In plasma FVIII binds with high affinity to von VWF, which protects it from premature catabolism and thus, plays in addition to its role in primary hemostasis a crucial role to regulate plasma levels of FVIII and as a consequence is also a central factor to control secondary hemostasis. The half-life of non-activated FVIII bound to VWF is about 12 to 14 hours in plasma. In von Willebrand disease type 3, where no or almost no VWF is present, the half-life of FVIII is only about 6 hours, leading to symptoms of mild to moderate hemophilia A in such patients due to decreased concentrations of FVIII. The stabilizing effect of VWF on FVIII has also been used to aid recombinant expression of FVIII in CHO cells (Kaufman et al. 1989, Mol Cell Biol).

There is a need for VWF molecules having improved affinity to FVIII in order to stabilize FVIII. It was surprisingly found that mutations in the D' domain of VWF can increase the affinity of VWF to FVIII. This allows providing FVIII/VWF complexes having a high affinity which are advantageous in therapy and prophylaxis of bleeding disorder.

SUMMARY OF THE INVENTION

In a first aspect the present invention relates to a polypeptide comprising a modified von Willebrand Factor (VWF),
wherein the amino acid sequence of said modified VWF has at least one mutation within the D' domain relative to the amino acid sequence of the D' domain of wild type VWF as shown in SEQ ID NO:31,
and wherein the binding affinity of said polypeptide comprising a modified VWF to Factor VIII (FVIII) is higher than that of a reference polypeptide, wherein the amino acid sequence of said reference polypeptide is identical to the amino acid sequence of said polypeptide comprising a modified VWF except that the amino acid sequence of the D' domain of the reference polypeptide consists of SEQ ID NO:31.

According to a preferred embodiment of the first aspect, the Factor VIII binding affinity of the polypeptide exceeds that of the reference polypeptide by at least 10 percent.

In another preferred embodiment, the affinity constant K_A for binding of the polypeptide to wild type Factor VIII is at least 3×10^{10} M^{-1}.

In one aspect, the invention relates to a polypeptide comprising a modified von Willebrand Factor (VWF),
wherein the amino acid sequence of said modified VWF has at least one substitution within the D' domain relative to the amino acid sequence of the D' domain of wild type VWF as shown in SEQ ID NO:31,
wherein said substitution replaces a negatively charged amino acid present in the D' domain of wild type VWF as shown in SEQ ID NO:31 with a neutral amino acid or with a positively charged amino acid.

In another aspect, the invention relates to a polypeptide comprising a modified von Willebrand Factor (VWF),
wherein the amino acid sequence of said modified VWF has at least one substitution within the D' domain relative to the amino acid sequence of the D' domain of wild type VWF as shown in SEQ ID NO:31,
wherein said substitution replaces a neutral amino acid present in the D' domain of wild type VWF as shown in SEQ ID NO:31 with a positively charged amino acid.

It is preferred that the mutation within the D' domain includes an amino acid substitution at one of positions 779, 781, 787, 789, 793, 794, 796, 798, 802, 818, 819, 825, 835, 838 and 853 of the VWF amino acid sequence as shown in SEQ ID NO:2.

It is further preferred that the mutation within the D' domain includes an amino acid substitution at one of positions 779, 781, 789, 793, 794, 802, 818, 819, 835, 838 and 853 of the VWF amino acid sequence as shown in SEQ ID NO:2. For example, the amino acid substitution within the D' domain may be selected from the group consisting of Asp779Asn, Leu781 Pro, Glu787Gln, Thr789Ala, Gln793Arg, Asn794Lys, Asp796Ala, Glu798Gln, Met802Arg, Met802Lys, Glu818Ala, Glu818Lys, Asn819Lys, Glu825Lys, Glu835Gln, Pro838Lys, and Asp853Asn, wherein the numbering refers to SEQ ID NO:2.

In another embodiment, the polypeptide comprises an amino acid sequence as shown in SEQ ID NO:33 or SEQ ID NO:34, with the proviso that the D' domain of the modified VWF contains at least one substitution relative to SEQ ID NO:31. For example, the polypeptide may comprise an amino acid sequence as shown in SEQ ID NO:35, with the proviso that the D' domain of the modified VWF contains at least one substitution relative to SEQ ID NO:31.

In another embodiment, the polypeptide of the present invention comprises an amino acid sequence as shown in SEQ ID NO:2 with the proviso that the D' domain of the modified VWF contains at least one amino acid substitution which increased FVIII binding by improving electrostatic attraction between the VWF polypeptide of the invention and FVIII, characterized in that acidic residues of the VWF D' domain are replaced by neutral or basic amino acids or neutral residues are replaced by basic amino acids.

In another preferred embodiment, the polypeptide of the present invention further comprises a half-life enhancing protein (HLEP). Preferrably, the HLEP is an albumin. The N-terminus of the albumin may be fused to the C-terminus of the VWF amino acid sequence.

A second aspect of the present invention is a complex comprising a Factor VIII molecule and a polypeptide of the present invention. Preferably, the complex has a dissociation constant K_D of 0.2 nmol/L or less. More preferably, the Factor VIII in the complex is the polypeptide of SEQ ID NO:37.

Yet another aspect of the present invention is the polypeptide of the present invention or the complex of the present invention for use in the treatment or prophylaxis of a bleeding disorder, e.g. of von Willebrand's disease (VWD) or hemophilia.

Yet another aspect of the present invention is a pharmaceutical composition comprising the polypeptide of the present invention or the complex of the present invention.

In another aspect, the invention relates to a method of treating a bleeding disorder, comprising administering to a patient in need thereof, a pharmaceutically effective amount of the polypeptide of the present invention or of the complex of the present invention. Preferably, the bleeding disorder is VWD or hemophilia A.

In yet another aspect the invention relates to a polynucleotide encoding the polypeptide of the present invention.

In another aspect, the invention pertains to a plasmid or vector comprising the polynucleotide of the present invention. The plasmid or vector is preferably an expression plasmid or expression vector.

In another aspect, the invention concerns a host cell comprising the polynucleotide or the plasmid of the present invention.

The invention further includes a method of producing a polypeptide comprising a modified VWF, comprising

(a) culturing the host cells of the present invention under conditions such that the polypeptide comprising a modified VWF is expressed; and

(b) optionally recovering the polypeptide comprising a modified VWF from the host cells or from the culture medium.

Yet another aspect of this invention is a method of increasing the Factor VIII binding affinity of VWF, comprising introducing a mutation into the D' domain of the VWF amino acid sequence, which is not present in the amino acid sequence of the D' domain of wild type VWF as shown in SEQ ID NO:31.

In another aspect, the invention relates to the use of a modified VWF having a higher affinity to FVIII than non-modified VWF for increasing the half-life of FVIII. The modified VWF is preferably a polypeptide of the invention as defined herein, or a modified VWF as defined herein. More preferably, the modified VWF is a fusion protein, most preferably an albumin fusion protein.

A further aspect of the invention is a method of preparing a complex comprising Factor VIII and VWF, said method comprising mixing a Factor VIII molecule with the polypeptide of the present invention or its half-life extended version.

DETAILED DESCRIPTION

The polypeptide of the present invention comprises a modified von Willebrand Factor.

VWF

The term "von Willebrand Factor" or "VWF", as used herein, refers to any polypeptide having the biological activity of wild type VWF. The gene encoding wild type VWF is transcribed into a 9 kb mRNA which is translated into a pre-propolypeptide of 2813 amino acids with an estimated molecular weight of 310,000 Da. The pre-propolypeptide contains a 22 amino acids signal peptide, a 741 amino acid pro-polypeptide and the mature subunit. Cleavage of the 741 amino acids propolypeptide from the N-terminus results in mature VWF consisting of 2050 amino acids. The amino acid sequence of the VWF pre-propolypeptide is shown in SEQ ID NO:2. Unless indicated otherwise, the amino acid numbering of VWF residues in this application refers to SEQ ID NO:2, even if the VWF molecule does not need to comprise all residues of SEQ ID NO:2. The amino acid sequence of mature VWF is shown in SEQ ID NO:32. The term "VWF" as used herein refers to the mature form of VWF unless indicated otherwise.

The propolypeptide of wild type VWF comprises multiple domains which are arranged in the following order:

D1-D2-D'-D3-A1-A2-A3-D4-B1-B2-B3-C1-C2-CK

The D1 and D2 domain represent the propeptide which is cleaved off to yield the mature VWF. The D' domain encompasses amino acids 764 to 865 of SEQ ID NO:2. The amino acid sequence of the D' domain of wild type VWF is shown in SEQ ID NO:31. The carboxylterminal 90 residues comprise the "CK" domain that is homologous to the "cystine knot" superfamily of protein. These family members have a tendency to dimerise through disulfide bonds.

Preferably, wild type VWF comprises the amino acid sequence of mature VWF as shown in SEQ ID NO:32. Also encompassed are additions, insertions, N-terminal, C-terminal or internal deletions of VWF as long as the biological activity of VWF is retained. The biological activity is retained in the sense of the invention if the VWF with deletions retains at least 10%, preferably at least 25%, more preferably at least 50%, most preferably at least 75% of the biological activity of wild-type VWF. The biological activity of wild-type VWF can be determined by the artisan using methods for ristocetin co-factor activity (Federici A B et al. 2004. Haematologica 89:77-85), binding of VWF to GP Ibα of the platelet glycoprotein complex Ib-V-IX (Sucker et al. 2006. Clin Appl Thromb Hemost. 12:305-310), or a collagen binding assay (Kallas & Talpsep. 2001. Annals of Hematology 80:466-471).

Factor VIII

The terms "blood coagulation Factor VIII", "Factor VIII" and "FVIII" are used interchangeably herein. "Blood coagulation Factor VIII" includes wild-type blood coagulation FVIII as well as derivatives of wild-type blood coagulation FVIII having the procoagulant activity of wild-type blood coagulation FVIII. Derivatives may have deletions, insertions and/or additions compared with the amino acid sequence of wild-type FVIII. The term FVIII includes proteolytically processed forms of FVIII, e.g. the form before activation, comprising heavy chain and light chain.

The term "FVIII" includes any FVIII variants or mutants having at least 25%, more preferably at least 50%, most preferably at least 75% of the biological activity of wild-type factor VIII.

As non-limiting examples, FVIII molecules include FVIII mutants preventing or reducing APC cleavage (Amano 1998. Thromb. Haemost. 79:557-563), FVIII mutants further stabilizing the A2 domain (WO 97/40145), FVIII mutants resulting in increased expression (Swaroop et al. 1997. JBC 272:24121-24124), FVIII mutants reducing its immunogenicity (Lollar 1999. Thromb. Haemost. 82:505-508), FVIII reconstituted from differently expressed heavy and light chains (Oh et al. 1999. Exp. Mol. Med. 31:95-100), FVIII mutants reducing binding to receptors leading to catabolism of FVIII like HSPG (heparan sulfate proteoglycans) and/or LRP (low density lipoprotein receptor related protein) (Ananyeva et al. 2001. TCM, 11:251-257), disulfide bond-stabilized FVIII variants (Gale et al., 2006. J. Thromb. Hemost. 4:1315-1322), FVIII mutants with improved secretion properties (Miao et al., 2004. Blood 103:3412-3419), FVIII mutants with increased cofactor specific activity (Wakabayashi et al., 2005. Biochemistry 44:10298-304), FVIII mutants with improved biosynthesis and secretion, reduced ER chaperone interaction, improved ER-Golgi transport, increased activation or resistance to inactivation and improved half-life (summarized by Pipe 2004. Sem. Thromb. Hemost. 30:227-237). All of these FVIII mutants and variants are incorporated herein by reference in their entirety.

Preferably FVIII comprises the full length sequence of FVIII as shown in SEQ ID NO:36. Also encompassed are additions, insertions, substitutions, N-terminal, C-terminal or internal deletions of FVIII as long as the biological activity of FVIII is retained. The biological activity is retained in the sense of the invention if the FVIII with modifications retains at least 10%, preferably at least 25%, more preferably at least 50%, most preferably at least 75%

of the biological activity of wild-type FVIII. The biological activity of FVIII can be determined by the artisan as described below.

A suitable test to determine the biological activity of FVIII is for example the one stage or the two stage coagulation assay (Rizza et al. 1982. Coagulation assay of FVIII:C and FIXa in Bloom ed. The Hemophilias. NY Churchchill Livingston 1992) or the chromogenic substrate FVIII:C assay (S. Rosen, 1984. Scand J Haematol 33: 139-145, suppl.). The content of these references is incorporated herein by reference.

The amino acid sequence of the mature wild-type form of human blood coagulation FVIII is shown in SEQ ID NO:36. The reference to an amino acid position of a specific sequence means the position of said amino acid in the FVIII wild-type protein and does not exclude the presence of mutations, e.g. deletions, insertions and/or substitutions at other positions in the sequence referred to. For example, a mutation in "Glu2004" referring to SEQ ID NO:36 does not exclude that in the modified homologue one or more amino acids at positions 1 through 2332 of SEQ ID NO:36 are missing.

"FVIII" and/or "VWF" within the above definition also include natural allelic variations that may exist and occur from one individual to another. "FVIII" and/or "VWF" within the above definition further includes variants of FVIII and or VWF. Such variants differ in one or more amino acid residues from the wild-type sequence. Examples of such differences may include as conservative amino acid substitutions, i.e. substitutions within groups of amino acids with similar characteristics, e.g. (1) small amino acids, (2) acidic amino acids, (3) polar amino acids, (4) basic amino acids, (5) hydrophobic amino acids, and (6) aromatic amino acids. Examples of such conservative substitutions are shown in the following table 1.

TABLE 1

(1)	Alanine	Glycine		
(2)	Aspartic acid	Glutamic acid		
(3)	Asparagine	Glutamine	Serine	Threonine
(4)	Arginine	Histidine	Lysine	
(5)	Isoleucine	Leucine	Methionine	Valine
(6)	Phenylalanine	Tyrosine	Tryptophane	

Modified VWF

The modified VWF of the present invention has an amino acid sequence which differs from that of wild-type VWF. According to the present invention the modified VWF has at least one mutation within its D' domain, as compared to the amino acid sequence of the D' domain of wild-type VWF as shown in SEQ ID NO:31. The mutation may be a deletion, insertion or substitution. Preferably, the mutation is an amino acid substitution.

The amino acid sequence of the D' domain of the modified VWF can have one or more mutations relative to SEQ ID NO:31. The amino acid sequence of the D' domain of the modified VWF may have one, two, three, four, five or more mutations relative to SEQ ID NO:31. It is preferred that the amino acid sequence of the D' domain of the modified VWF has one, two or three mutations relative to SEQ ID NO:31. Most preferably, the amino acid sequence of the D' domain of the modified VWF has exactly one substitution relative to the amino acid sequence as shown in SEQ ID NO:31.

In a first approach, the amino acid positions which are preferably mutated in the modified VWF increase the positive charge of the D' domain and/or reduce the negative charge thereof. This first approach is referred to herein as "electrostatic approach". This can be achieved by replacing at least one amino acid having a negative charge at pH 7.4 with at least one amino acid which is neutral or has a positive charge at pH 7.4. Alternatively, this can be achieved by replacing at least one amino acid which is neutral at pH 7.4 with at least one amino acid having a positive charge at pH 7.4. These amino acid types are defined as follows:

- Amino acids having a negative charge at pH 7.4 are aspartic acid (aspartate) and glutamic acid (glutamate); they are referred to as "negatively charged amino acids" hereinafter.
- Amino acids having a positive charge at pH 7.4 are lysine and arginine; they are referred to as "positively charged amino acids" hereinafter.
- Amino acids which are neutral at pH 7.4 are alanine, glycine, asparagine, glutamine, serine, threonine, histidine, isoleucine, leucine, methionine, valine, phenylalanine, tyrosine, tryptophane, proline, and cysteine; they are referred to as "neutral amino acids" hereinafter.

In one aspect of the electrostatic approach, the invention relates to a polypeptide comprising a modified von Willebrand Factor (VWF),

wherein the amino acid sequence of said modified VWF has at least one substitution within the D' domain relative to the amino acid sequence of the D' domain of wild type VWF as shown in SEQ ID NO:31,

wherein said substitution replaces a negatively charged amino acid present in the D' domain of wild type VWF as shown in SEQ ID NO:31 with a neutral amino acid or with a positively charged amino acid.

In another aspect of the electrostatic approach, the invention relates to a polypeptide comprising a modified von Willebrand Factor (VWF),

wherein the amino acid sequence of said modified VWF has at least one substitution within the D' domain relative to the amino acid sequence of the D' domain of wild type VWF as shown in SEQ ID NO:31,

wherein said substitution replaces a neutral amino acid present in the D' domain of wild type VWF as shown in SEQ ID NO:31 with a positively charged amino acid.

The modified VWF of the present invention includes, but is not limited to, the following embodiments in accordance with the electrostatic approach which can be combined with each other.

- In a first embodiment in accordance with the electrostatic approach, the amino acid at position 779 of the VWF amino acid sequence is a neutral amino acid or a positively charged amino acid. The amino acid at position 779 of the VWF amino acid sequence may be lysine. Alternatively, the amino acid at position 779 of the VWF amino acid sequence may be arginine. Alternatively, the amino acid at position 779 of the VWF amino acid sequence may be a neutral amino acid.
- In a second embodiment in accordance with the electrostatic approach, the amino acid at position 787 of the VWF amino acid sequence is a neutral amino acid or a positively charged amino acid. The amino acid at position 787 of the VWF amino acid sequence may be lysine. Alternatively, the amino acid at position 787 of the VWF amino acid sequence may be arginine. Alternatively, the amino acid at position 787 of the VWF amino acid sequence may be a neutral amino acid.
- In a third embodiment in accordance with the electrostatic approach, the amino acid at position 793 of the VWF amino acid sequence is a positively charged amino acid. The amino acid at position 793 of the VWF amino

acid sequence may be lysine. Alternatively, the amino acid at position 793 of the VWF amino acid sequence may be arginine.

In a fourth embodiment in accordance with the electrostatic approach, the amino acid at position 794 of the VWF amino acid sequence is a positively charged amino acid. The amino acid at position 794 of the VWF amino acid sequence may be lysine. Alternatively, the amino acid at position 794 of the VWF amino acid sequence may be arginine.

In a fifth embodiment in accordance with the electrostatic approach, the amino acid at position 796 of the VWF amino acid sequence is a neutral amino acid or a positively charged amino acid. The amino acid at position 796 of the VWF amino acid sequence may be lysine. Alternatively, the amino acid at position 796 of the VWF amino acid sequence may be arginine. Alternatively, the amino acid at position 796 of the VWF amino acid sequence may be a neutral amino acid.

In a sixth embodiment in accordance with the electrostatic approach, the amino acid at position 798 of the VWF amino acid sequence is a neutral amino acid or a positively charged amino acid. The amino acid at position 798 of the VWF amino acid sequence may be lysine. Alternatively, the amino acid at position 798 of the VWF amino acid sequence may be arginine. Alternatively, the amino acid at position 798 of the VWF amino acid sequence may be a neutral amino acid.

In a seventh embodiment in accordance with the electrostatic approach, the amino acid at position 802 of the VWF amino acid sequence is a positively charged amino acid. The amino acid at position 802 of the VWF amino acid sequence may be lysine. Alternatively, the amino acid at position 802 of the VWF amino acid sequence may be arginine.

In an eighth embodiment in accordance with the electrostatic approach, the amino acid at position 818 of the VWF amino acid sequence is a neutral amino acid or a positively charged amino acid. The amino acid at position 818 of the VWF amino acid sequence may be lysine. Alternatively, the amino acid at position 818 of the VWF amino acid sequence may be arginine. Alternatively, the amino acid at position 818 of the VWF amino acid sequence may be a neutral amino acid.

In a ninth embodiment in accordance with the electrostatic approach, the amino acid at position 819 of the VWF amino acid sequence is a positively charged amino acid. The amino acid at position 819 of the VWF amino acid sequence may be lysine. Alternatively, the amino acid at position 819 of the VWF amino acid sequence may be arginine.

In a tenth embodiment in accordance with the electrostatic approach, the amino acid at position 825 of the VWF amino acid sequence is a neutral amino acid or a positively charged amino acid. The amino acid at position 825 of the VWF amino acid sequence may be lysine. Alternatively, the amino acid at position 825 of the VWF amino acid sequence may be arginine. Alternatively, the amino acid at position 825 of the VWF amino acid sequence may be a neutral amino acid.

In an eleventh embodiment in accordance with the electrostatic approach, the amino acid at position 835 of the VWF amino acid is a neutral amino acid or a positively charged amino acid. The amino acid at position 835 of the VWF amino acid sequence may be lysine. Alternatively, the amino acid at position 835 of the VWF amino acid sequence may be arginine. Alternatively, the amino acid at position 835 of the VWF amino acid sequence may be a neutral amino acid.

In a twelfth embodiment in accordance with the electrostatic approach, the amino acid at position 838 of the VWF amino acid sequence is a positively charged amino acid. The amino acid at position 838 of the VWF amino acid sequence may be lysine. Alternatively, the amino acid at position 838 of the VWF amino acid sequence may be arginine.

In a thirteenth embodiment, the amino acid at position 853 of the VWF amino acid sequence is a neutral amino acid or a positively charged amino acid. The amino acid at position 853 of the VWF amino acid sequence may be lysine. Alternatively, the amino acid at position 853 of the VWF amino acid sequence may be arginine. Alternatively, the amino acid at position 853 of the VWF amino acid sequence may be a neutral amino acid.

In an alternative second approach, an amino acid in the D' domain may be replaced with a different amino acid which is evolutionarily conserved or at least polymorphic at the respective position. This second approach is referred to as "evolutionary approach" hereinafter.

Replacing with a different amino acid which is evolutionarily conserved in the sense of the invention means that a given amino acid within the D' domain which is present (i) only in humans is replaced with a different amino acid which is conserved in other species at the respective amino acid position, or (ii) which is present only in humans and some other species but not in most species is replaced with the more abundant respective amino acid which is present in most other species, or (iii) is common human polymorphism. This means that at least one residue of the VWF D' domain is replaced with a different amino acid which is present at the same position in one or more VWF polymorphs or orthologues having a D' domain different from SEQ ID NO:31.

The modified VWF of the present invention includes, but is not limited to, the following embodiments in accordance with the evolutionary approach which can be combined with each other and with any embodiment(s) of the electrostatic approach.

In a first embodiment in accordance with the evolutionary approach, the amino acid at position 781 of the VWF amino acid sequence is a proline.

In a second embodiment in accordance with the evolutionary approach, the amino acid at position 789 of the VWF amino acid sequence is alanine, glycine, serine or valine.

In other preferred embodiments of the invention the D' domain of the modified VWF has the following sequence (SEQ ID NO:33).

SLSCRPPMVK LVCPAX^{1}NX^{2}RA EGLX^{3}CX^{4}KTCX^{5} X^{6}YX^{7}LX^{8}CMSX^{9}G CVSGCLCPPG MVRHX^{10}X^{11}RCVA LX^{12}RCPCFHQG KX^{13}YAX^{14}GETVK IGCNTCVCRX^{15} RKWNCTDHVC DA

The modified D' domain in the polypeptide of the present invention may have an amino acid sequence in accordance with one of the embodiments in the following table 2. Each line with an "embodiment No." represents an embodiment, wherein the D' domain in the polypeptide of the present invention has the amino acid sequence as shown in SEQ ID NO:33 with X^{1} through X^{15} having the indicated meanings. "neut" means a neutral amino acid.

TABLE 2

Embodiment No.	X^1	X^2	X^3	X^4	X^5	X^6	X^7	X^8	X^9	X^{10}	X^{11}	X^{12}	X^{13}	X^{14}	X^{15}
Wild type (SEQ ID NO: 31)	D	L	E	T	Q	N	D	E	M	E	N	E	E	P	D
1.1 (SEQ ID NO: 33)	neut	L	E	T	Q	N	D	E	M	E	N	E	E	P	D
1.2 (SEQ ID NO: 33)	K	L	E	T	Q	N	D	E	M	E	N	E	E	P	D
1.3 (SEQ ID NO: 33)	R	L	E	T	Q	N	D	E	M	E	N	E	E	P	D
2.1 (SEQ ID NO: 33)	D	P	E	T	Q	N	D	E	M	E	N	E	E	P	D
3.1 (SEQ ID NO: 33)	D	L	neut	T	Q	N	D	E	M	E	N	E	E	P	D
3.2 (SEQ ID NO: 33)	D	L	K	T	Q	N	D	E	M	E	N	E	E	P	D
3.3 (SEQ ID NO: 33)	D	L	R	T	Q	N	D	E	M	E	N	E	E	P	D
4.1 (SEQ ID NO: 33)	D	L	E	A	Q	N	D	E	M	E	N	E	E	P	D
4.2 (SEQ ID NO: 33)	D	L	E	G	Q	N	D	E	M	E	N	E	E	P	D
4.3 (SEQ ID NO: 33)	D	L	E	S	Q	N	D	E	M	E	N	E	E	P	D
4.4 (SEQ ID NO: 33)	D	L	E	V	Q	N	D	E	M	E	N	E	E	P	D
5.1 (SEQ ID NO: 33)	D	L	E	T	K	N	D	E	M	E	N	E	E	P	D
5.2 (SEQ ID NO: 33)	D	L	E	T	R	N	D	E	M	E	N	E	E	P	D
6.1 (SEQ ID NO: 33)	D	L	E	T	Q	K	D	E	M	E	N	E	E	P	D
6.2 (SEQ ID NO: 33)	D	L	E	T	Q	R	D	E	M	E	N	E	E	P	D
7.1 (SEQ ID NO: 33)	D	L	E	T	Q	N	neut	E	M	E	N	E	E	P	D
7.2 (SEQ ID NO: 33)	D	L	E	T	Q	N	K	E	M	E	N	E	E	P	D
7.3 (SEQ ID NO: 33)	D	L	E	T	Q	N	R	E	M	E	N	E	E	P	D
8.1 (SEQ ID NO: 33)	D	L	E	T	Q	N	D	neut	M	E	N	E	E	P	D
8.2 (SEQ ID NO: 33)	D	L	E	T	Q	N	D	K	M	E	N	E	E	P	D
8.3 (SEQ ID NO: 33)	D	L	E	T	Q	N	D	R	M	E	N	E	E	P	D
9.1 (SEQ ID NO: 33)	D	L	E	T	Q	N	D	E	K	E	N	E	E	P	D
9.2 (SEQ ID NO: 33)	D	L	E	T	Q	N	D	E	R	E	N	E	E	P	D
10.1 (SEQ ID NO: 33)	D	L	E	T	Q	N	D	E	M	neut	N	E	E	P	D

TABLE 2-continued

Embodiment No.	X^1	X^2	X^3	X^4	X^5	X^6	X^7	X^8	X^9	X^{10}	X^{11}	X^{12}	X^{13}	X^{14}	X^{15}
10.2 (SEQ ID NO: 33)	D	L	E	T	Q	N	D	E	M	K	N	E	E	P	D
10.3 (SEQ ID NO: 33)	D	L	E	T	Q	N	D	E	M	R	N	E	E	P	D
11.1 (SEQ ID NO: 33)	D	L	E	T	Q	N	D	E	M	E	K	E	E	P	D
11.2 (SEQ ID NO: 33)	D	L	E	T	Q	N	D	E	M	E	R	E	E	P	D
12.1 (SEQ ID NO: 33)	D	L	E	T	Q	N	D	E	M	E	N	neut	E	P	D
12.2 (SEQ ID NO: 33)	D	L	E	T	Q	N	D	E	M	E	N	K	E	P	D
12.3 (SEQ ID NO: 33)	D	L	E	T	Q	N	D	E	M	E	N	R	E	P	D
13.1 (SEQ ID NO: 33)	D	L	E	T	Q	N	D	E	M	E	N	E	neut	P	D
13.2 (SEQ ID NO: 33)	D	L	E	T	Q	N	D	E	M	E	N	E	K	P	D
13.3 (SEQ ID NO: 33)	D	L	E	T	Q	N	D	E	M	E	N	E	R	P	D
14.1 (SEQ ID NO: 33)	D	L	E	T	Q	N	D	E	M	E	N	E	E	K	D
14.2 (SEQ ID NO: 33)	D	L	E	T	Q	N	D	E	M	E	N	E	E	R	D
15.1 (SEQ ID NO: 33)	D	L	E	T	Q	N	D	E	M	E	N	E	E	P	neut
15.2 (SEQ ID NO: 33)	D	L	E	T	Q	N	D	E	M	E	N	E	E	P	K
15.3 (SEQ ID NO: 33)	D	L	E	T	Q	N	D	E	M	E	N	E	E	P	R

Embodiments 2.1 and 4.1-4.4 of table 2 are in accordance with the evolutionary approach, all other embodiments are in accordance with the electrostatic approach.

The amino acid positions which are preferably mutated in the modified VWF are selected from the group consisting of amino acid positions 779, 781, 787, 793, 794, 796, 798, 802, 818, 819, 825, 835, 838 and 853, wherein the numbering refers to the amino acid sequence shown in SEQ ID NO:2. That is, the D' domain of the modified VWF preferably has an amino acid substitution at one of positions 16, 18, 26, 30, 31, 39, 55, 56, 72, 75 or 90 of SEQ ID NO:31.

Preferably, the amino acid substitution in the modified VWF is at one of positions 789, 802, 818, 819 or 853 of the amino acid sequence as shown in SEQ ID NO:2. That is, the D' domain of the modified VWF preferably has one or more mutations at positions 39, 55, 56 and 90 of SEQ ID NO:31.

According to this invention the binding affinity of the polypeptide of the present invention to FVIII is higher than that of a reference polypeptide which has the same amino acid sequence except for the mutation in the D' domain.

The binding affinity of a VWF molecule to a Factor VIII molecule can be determined by a binding assay used in the art. For example, the VWF molecule may be immobilized on a solid support, increasing concentrations of Factor VIII are applied, incubated for a certain period of time, and after washing, bound Factor VIII is determined with a chromogenic assay. The affinity constant or dissociation constant may then be determined by Scatchard analysis or another suitable method. A method of determining the affinity of binding of human Factor VIII to von Willebrand Factor are described in Vlot et al. (1995), Blood, Volume 85, Number 11, 3150-3157. Preferably, however, the affinity of VWF to Factor VIII is determined as described in Example 4 of this application.

Any indication herein of affinity, including dissociation constants, preferably refers to the binding of the modified VWF of the invention, or of the polypeptide of the invention to single chain FVIII represented by the amino acid sequence as shown in SEQ ID NO:37.

The dissociation constant of the complex consisting of VWF and FVIII is preferably 0.2 nmol/L or less, more preferably 0.175 nmol/L or less, more preferably 0.15 nmol/L or less, more preferably 0.125 nmol/L or less, more preferably 0.1 nmol/L or less, more preferably 0.05 nmol/L or less, most preferably 0.01 nmol/L or less.

The dissociation constant K_D of a complex of the polypeptide of the invention and the Factor VIII of SEQ ID NO:37 is typically less than 90% of the dissociation constant

K_D of a complex of the reference polypeptide (e.g. the polypeptide of SEQ ID NO:32) and the Factor VIII of SEQ ID NO:37. The dissociation constant K_D of a complex of the polypeptide of the invention and the Factor VIII of SEQ ID NO:37 is preferably less than 75%, more preferably less than 50%, more preferably less than 25%, more preferably less than 10%, more preferably less than 5%, of the dissociation constant K_D of a complex of the reference polypeptide (e.g. the polypeptide of SEQ ID NO:32) and the Factor VIII of SEQ ID NO:37.

The binding affinity of the polypeptide of the present invention comprising the modified VWF to Factor VIII exceeds that of the reference polypeptide by at least 10%, preferably by at least 20%, more preferably by at least 30%, most preferably by at least 50%, more preferably by at least 75%, more preferably by at least 100%, more preferably by at least 250%, more preferably by at least 500%, more preferably by at least 1000%, more preferably by at least 10000%, most preferably by at least 100000%.

It has been found that the affinity of the polypeptide of the invention to single chain Factor VIII (e.g. represented by SEQ ID NO:37) is higher than to heterodimeric "two-chain" Factor VIII (e.g. represented by SEQ ID NO:36). Therefore, the preferred Factor VIII molecule in the complex of the invention is a single chain Factor VIII, most preferably it is the polypeptide of SEQ ID NO:37.

The reference polypeptide is a polypeptide the amino acid sequence of which is identical to that of the polypeptide of the present invention except for the mutation within the D' domain of VWF. That is, the reference polypeptide preferably has an amino acid sequence identical to that of the polypeptide of the present invention, with the proviso that the D' domain in the reference polypeptide consists of the amino acid sequence as shown in SEQ ID NO:31. In other words, the only difference in sequence between the polypeptide of the invention and the reference polypeptide lies in the amino acid sequence of the D' domain. The reference polypeptide has preferably been prepared under the same conditions as the polypeptide of the invention.

The polypeptide of the present invention may consist of the modified VWF. In another embodiment, the polypeptide of the present invention comprises a further amino acid sequence, preferably a heterologous amino acid sequence. The heterologous amino acid sequence is typically not fused to VWF in nature.

The present invention is particularly useful in cases where a VWF variant is used having an improved half-life. This can be achieved for example by fusing VWF to human serum albumin. It has been found, however, that such fusion proteins may have a reduced affinity to FVIII as compared to wild type VWF. This includes the risk that a complex of VWF fusion protein and FVIII administered to a patient may dissociate rather quickly, and the FVIII dissociated from the complex would bind to endogenous VWF. The positive effect of the complexation between a VWF with an increased half-life and FVIII, namely that also the half-life of FVIII is increased, can thus be lost if the affinity between VWF fusion protein and FVIII is too low. This problem is addressed by improving the binding of VWF to FVIII in accordance with this invention. As VWF fusion proteins are particularly at risk of having a reduced FVIII affinity, the present invention is particularly applicable to VWF fusion proteins.

Therefore, in one embodiment, the polypeptide of the present invention comprises the modified VWF and a half-life enhancing protein (HLEP). Preferably, the HLEP is an albumin.

One or more HLEPs may be fused to the C-terminal part of VWF preferably as not to interfere with the binding capabilities of VWF for example to FVIII, platelets, heparin or collagen.

In one embodiment the modified VWF has the following structure:

N-VWF-C-L1-H, [formula 1]

wherein

N is an N-terminal part of VWF,

L1 is a chemical bond or a linker sequence

H is a HLEP, and

C is a C-terminal part of VWF

L1 may be a chemical bond or a linker sequence consisting of one or more amino acids, e.g. of 1 to 50, 1 to 30, 1 to 20, 1 to 15, 1 to 10, 1 to 5 or 1 to 3 (e.g. 1, 2 or 3) amino acids and which may be equal or different from each other. Usually, the linker sequences are not present at the corresponding position in the wild-type coagulation factor. Examples of suitable amino acids present in L1 include Gly and Ser.

Preferred HLEP sequences are described infra. Likewise encompassed by the invention are fusions to the exact "N-terminal amino acid" of the respective HLEP, or fusions to the "N-terminal part" of the respective HLEP, which includes N-terminal deletions of one or more amino acids of the HLEP.

The modified VWF or the complex of the FVIII with the modified VWF of the invention may comprise more than one HLEP sequence, e.g. two or three HLEP sequences. These multiple HLEP sequences may be fused to the C-terminal part of VWF in tandem, e.g. as successive repeats.

Linker Sequences

According to this invention, the therapeutic polypeptide moiety may be coupled to the HLEP moiety by a peptide linker. The linker should be non-immunogenic and may be a non-cleavable or cleavable linker.

Non-cleavable linkers may be comprised of alternating glycine and serine residues as exemplified in WO2007/090584.

In another embodiment of the invention the peptidic linker between the FVIII and/or the VWF moiety and the albumin moiety consists of peptide sequences, which serve as natural interdomain linkers in human proteins. Preferably such peptide sequences in their natural environment are located close to the protein surface and are accessible to the immune system so that one can assume a natural tolerance against this sequence. Examples are given in WO2007/090584.

Cleavable linkers should be flexible enough to allow cleavage by proteases. In a preferred embodiment the cleavage of the linker proceeds comparably fast as the activation of FVIII within the fusion protein, if the fusion protein is a modified FVIII.

The cleavable linker preferably comprises a sequence derived from

a) the therapeutic polypeptide to be administered itself if it contains proteolytic cleavage sites that are proteolytically cleaved during activation of the therapeutic polypeptide,

b) a substrate polypeptide cleaved by a protease which is activated or formed by the involvement of the therapeutic polypeptide.

c) a polypeptide involved in coagulation or fibrinolysis

The linker region in a more preferred embodiment comprises a sequence of FVIII and/or VWF, which should result in a decreased risk of neoantigenic properties of the expressed fusion protein. Also in case the therapeutic protein

is FVIII which needs to be proteolytically activated, the kinetics of the peptide linker cleavage will more closely reflect the coagulation-related activation kinetics of the zymogen.

The linker peptides are preferably cleavable by the proteases of the coagulation system, for example FIIa, FIXa, FXa, FXIa, FXIIa and FVIIa.

Exemplary combinations of therapeutic polypeptide, cleavable linker and HLEP include the constructs listed in WO2007/090584 (for example in table 2 and figure 4) and WO2007/144173 (for example in table 3a and 3b), but are not limited to these.

Half-Life Enhancing Polypeptides (HLEPs)

A "half-life enhancing polypeptide" as used herein is selected from the group consisting of albumin, a member of the albumin-family, the constant region of immunoglobulin G and fragments thereof, region and polypeptides capable of binding under physiological conditions to albumin, to members of the albumin family as well as to portions of an immunoglobulin constant region. It may be a full-length half-life-enhancing protein described herein (e.g. albumin, a member of the albumin-family or the constant region of immunoglobulin G) or one or more fragments thereof that are capable of stabilizing or prolonging the therapeutic activity or the biological activity of the coagulation factor. Such fragments may be of 10 or more amino acids in length or may include at least about 15, at least about 20, at least about 25, at least about 30, at least about 50, at least about 100, or more contiguous amino acids from the HLEP sequence or may include part or all of specific domains of the respective HLEP, as long as the HLEP fragment provides a functional half-life extension of at least 25% compared to a wild-type VWF.

The HLEP portion of the proposed coagulation factor insertion constructs of the invention may be a variant of a normal HLEP. The term "variants" includes insertions, deletions and substitutions, either conservative or non-conservative, where such changes do not substantially alter the active site, or active domain which confers the biological activities of the modified VWF.

In particular, the proposed VWF HLEP fusion constructs of the invention may include naturally occurring polymorphic variants of HLEPs and fragments of HLEPs. The HLEP may be derived from any vertebrate, especially any mammal, for example human, monkey, cow, sheep, or pig. Non-mammalian HLEPs include, but are not limited to, hen and salmon.

Albumin as HLEP

The terms, "human serum albumin" (HSA) and "human albumin" (HA) and "albumin" (ALB) are used interchangeably in this application. The terms "albumin" and "serum albumin" are broader, and encompass human serum albumin (and fragments and variants thereof) as well as albumin from other species (and fragments and variants thereof).

As used herein, "albumin" refers collectively to albumin polypeptide or amino acid sequence, or an albumin fragment or variant, having one or more functional activities (e.g., biological activities) of albumin. In particular, "albumin" refers to human albumin or fragments thereof, especially the mature form of human albumin as shown in SEQ ID NO:38 herein or albumin from other vertebrates or fragments thereof, or analogs or variants of these molecules or fragments thereof.

In particular, the proposed VWF fusion constructs of the invention may include naturally occurring polymorphic variants of human albumin and fragments of human albumin. Generally speaking, an albumin fragment or variant will be at least 10, preferably at least 40, most preferably more than 70 amino acids long. The albumin variant may preferentially consist of or alternatively comprise at least one whole domain of albumin or fragments of said domains, for example domains 1 (amino acids 1-194 of SEQ ID NO:38), 2 (amino acids 195-387 of SEQ ID NO: 38), 3 (amino acids 388-585 of SEQ ID NO: 38), 1+2 (1-387 of SEQ ID NO: 38), 2+3 (195-585 of SEQ ID NO: 38) or 1+3 (amino acids 1-194 of SEQ ID NO: 38+amino acids 388-585 of SEQ ID NO: 38). Each domain is itself made up of two homologous subdomains namely 1-105, 120-194, 195-291, 316-387, 388-491 and 512-585, with flexible inter-subdomain linker regions comprising residues Lys106 to Glu119, Glu292 to Val315 and Glu492 to Ala511.

The albumin portion of the proposed VWF fusion constructs of the invention may comprise at least one subdomain or domain of HA or conservative modifications thereof.

In a preferred embodiment the N-terminus of albumin is fused to the C-terminus of the amino acid sequence of the modified VWF. That is, the polypeptide of the present invention may have the structure:

N-mVWF-C-L1-A,

wherein N is an N-terminal part of VWF, mVWF is the modified VWF as described hereinabove, C is a C-terminal part of VWF, L1 is a chemical bond or a linker sequence and A is albumin as defined hereinabove.

Immunoglobulins as HLEPs

Immunoglobulin G (IgG) constant regions (Fc) are known in the art to increase the half-life of therapeutic proteins (Dumont J A et al. 2006. BioDrugs 20:151-160). The IgG constant region of the heavy chain consists of 3 domains (CH1-CH3) and a hinge region. The immunoglobulin sequence may be derived from any mammal, or from subclasses IgG1, IgG2, IgG3 or IgG4, respectively. IgG and IgG fragments without an antigen-binding domain may also be used as HLEPs. The therapeutic polypeptide portion is connected to the IgG or the IgG fragments preferably via the hinge region of the antibody or a peptidic linker, which may even be cleavable. Several patents and patent applications describe the fusion of therapeutic proteins to immunoglobulin constant regions to enhance the therapeutic protein's in vivo half-lifes. US 2004/0087778 and WO 2005/001025 describe fusion proteins of Fc domains or at least portions of immunoglobulin constant regions with biologically active peptides that increase the half-life of the peptide, which otherwise would be quickly eliminated in vivo. Fc-IFN-β fusion proteins were described that achieved enhanced biological activity, prolonged circulating half-life and greater solubility (WO 2006/000448). Fc-EPO proteins with a prolonged serum half-life and increased in vivo potency were disclosed (WO 2005/063808) as well as Fc fusions with G-CSF (WO 2003/076567), glucagon-like peptide-1 (WO 2005/000892), clotting factors (WO 2004/101740) and interleukin-10 (U.S. Pat. No. 6,403,077), all with half-life enhancing properties.

In another embodiment, the functional half-life of polypeptide of the invention or of FVIII complexed with the polypeptide of the invention is prolonged compared to that of wild type VWF or to that of FVIII complexed with wild type VWF, or with the reference polypeptide as defined supra. The increase may be more than 15%, for example at least 20% or at least 50%. Again, such functional half-life values can be measured in vitro in blood samples taken at

different time intervals from said mammal after the modified VWF or the complex of FVIII with modified VWF has been administered.

In another embodiment of the invention, the polypeptide of the invention or FVIII complexed with the polypeptide of the invention exhibits an improved in vivo recovery compared to wild type VWF or to FVIII complexed with wild type VWF, or with the reference polypeptide defined supra. The in vivo recovery can be determined in vivo for example in normal animals or in animal models of hemophilia A, like FVIII knockout mice in which one would expect an increased percentage of FVIII be found by antigen or activity assays in the circulation shortly (5 to 10 min.) after i.v. administration compared to the corresponding wild-type VWF, or reference polypeptide defined supra.

The in vivo recovery is preferably increased by at least 10%, more preferably by at least 20%, and even more preferably by at least 40% compared to FVIII complexed with wild-type VWF, or with the reference polypeptide defined supra.

In yet another embodiment of the invention immunoglobulin constant regions or portions thereof are used as HLEPs. Preferably the Fc region comprised of a CH2 and CH3 domain and a hinge region of an IgG, more preferably of an IgG1 or fragments or variants thereof are used, variants including mutations which enhance binding to the neonatal Fc receptor (FcRn).

Polynucleotides

The invention further relates to a polynucleotide encoding a modified VWF or a polypeptide comprising said modified VWF, as described in this application. The term "polynucleotide(s)" generally refers to any polyribonucleotide or polydeoxyribonucleotide that may be unmodified RNA or DNA or modified RNA or DNA. The polynucleotide may be single- or double-stranded DNA, single or double-stranded RNA. As used herein, the term "polynucleotide(s)" also includes DNAs or RNAs that comprise one or more modified bases and/or unusual bases, such as inosine. It will be appreciated that a variety of modifications may be made to DNA and RNA that serve many useful purposes known to those of skill in the art. The term "polynucleotide(s)" as it is employed herein embraces such chemically, enzymatically or metabolically modified forms of polynucleotides, as well as the chemical forms of DNA and RNA characteristic of viruses and cells, including, for example, simple and complex cells.

The skilled person will understand that, due to the degeneracy of the genetic code, a given polypeptide can be encoded by different polynucleotides. These "variants" are encompassed by this invention.

Preferably, the polynucleotide of the invention is an isolated polynucleotide. The term "isolated" polynucleotide refers to a polynucleotide that is substantially free from other nucleic acid sequences, such as and not limited to other chromosomal and extrachromosomal DNA and RNA. Isolated polynucleotides may be purified from a host cell. Conventional nucleic acid purification methods known to skilled artisans may be used to obtain isolated polynucleotides. The term also includes recombinant polynucleotides and chemically synthesized polynucleotides.

The invention further relates to a group of polynucleotides which together encode the modified VWF of the invention, or the polypeptide of the invention comprising the modified VWF. A first polynucleotide in the group may encode the N-terminal part of the modified VWF, and a second polynucleotide may encode the C-terminal part of the modified VWF.

Yet another aspect of the invention is a plasmid or vector comprising a polynucleotide according to the invention. Preferably, the plasmid or vector is an expression vector. In a particular embodiment, the vector is a transfer vector for use in human gene therapy.

The invention also relates to a group of plasmids or vectors that comprise the above group of polynucleotides. A first plasmid or vector may contain said first polynucleotide, and a second plasmid or vector may contain said second polynucleotide. Alternatively, both coding sequences are cloned into one expression vector either using two separate promoter sequences or one promoter and an internal ribosome entry site (IRES) element which may be used for example to direct the expression furin to enhance the generation of mature VWF.

Still another aspect of the invention is a host cell comprising a polynucleotide, a plasmid or vector of the invention, or a group of polynucleotides or a group of plasmids or vectors as described herein.

The host cells of the invention may be employed in a method of producing a modified VWF or a polypeptide comprising said modified VWF, which is part of this invention. The method comprises:

(a) culturing host cells of the invention under conditions such that the desired modified protein is expressed; and
(b) optionally recovering the desired modified protein from the host cells or from the culture medium.

It is preferred to purify the modified VWF of the present invention, or the polypeptide comprising the modified VWF to ≥80% purity, more preferably ≥95% purity, and particularly preferred is a pharmaceutically pure state that is greater than 99.9% pure with respect to contaminating macromolecules, particularly other proteins and nucleic acids, and free of infectious and pyrogenic agents. Preferably, an isolated or purified modified modified VWF of the invention or polypeptide of the invention is substantially free of other, non-related polypeptides.

The various products of the invention are useful as medicaments. Accordingly, the invention relates to a pharmaceutical composition comprising a modified VWF or a polypeptide comprising said modified VWF as described herein, a polynucleotide of the invention, or a plasmid or vector of the invention.

The invention also concerns a method of treating an individual suffering from a blood coagulation disorder such as hemophilia A or B or VWD. The method comprises administering to said individual an efficient amount of (i) FVIII and of the modified VWF or the polypeptide comprising the modified VWF or (ii) of the complex of FVIII with modified VWF or (iii) of the complex of FVIII with the polypeptide comprising modified VWF as described herein. In another embodiment, the method comprises administering to the individual an efficient amount of a polynucleotide of the invention or of a plasmid or vector of the invention. Alternatively, the method may comprise administering to the individual an efficient amount of the host cells of the invention described herein.

Expression of the Proposed Mutants

The production of recombinant mutant proteins at high levels in suitable host cells requires the assembly of the above-mentioned modified cDNAs into efficient transcriptional units together with suitable regulatory elements in a recombinant expression vector that can be propagated in various expression systems according to methods known to those skilled in the art. Efficient transcriptional regulatory elements could be derived from viruses having animal cells as their natural hosts or from the chromosomal DNA of

animal cells. Preferably, promoter-enhancer combinations derived from the Simian Virus 40, adenovirus, BK polyoma virus, human cytomegalovirus, or the long terminal repeat of Rous sarcoma virus, or promoter-enhancer combinations including strongly constitutively transcribed genes in animal cells like beta-actin or GRP78 can be used. In order to achieve stable high levels of mRNA transcribed from the cDNAs, the transcriptional unit should contain in its 3'-proximal part a DNA region encoding a transcriptional termination-polyadenylation sequence. Preferably, this sequence is derived from the Simian Virus 40 early transcriptional region, the rabbit beta-globin gene, or the human tissue plasminogen activator gene.

The cDNAs are then integrated into the genome of a suitable host cell line for expression of the modified FVIII and/or VWF proteins. Preferably this cell line should be an animal cell-line of vertebrate origin in order to ensure correct folding, disulfide bond formation, asparagine-linked glycosylation and other post-translational modifications as well as secretion into the cultivation medium. Examples on other post-translational modifications are tyrosine O-sulfation and proteolytic processing of the nascent polypeptide chain. Examples of cell lines that can be use are monkey COS-cells, mouse L-cells, mouse C127-cells, hamster BHK-21 cells, human embryonic kidney 293 cells, and hamster CHO-cells.

The recombinant expression vector encoding the corresponding cDNAs can be introduced into an animal cell line in several different ways. For instance, recombinant expression vectors can be created from vectors based on different animal viruses. Examples of these are vectors based on baculovirus, vaccinia virus, adenovirus, and preferably bovine papilloma virus.

The transcription units encoding the corresponding DNA's can also be introduced into animal cells together with another recombinant gene which may function as a dominant selectable marker in these cells in order to facilitate the isolation of specific cell clones which have integrated the recombinant DNA into their genome. Examples of this type of dominant selectable marker genes are Tn5 amino glycoside phosphotransferase, conferring resistance to geneticin (G418), hygromycin phosphotransferase, conferring resistance to hygromycin, and puromycin acetyl transferase, conferring resistance to puromycin. The recombinant expression vector encoding such a selectable marker can reside either on the same vector as the one encoding the cDNA of the desired protein, or it can be encoded on a separate vector which is simultaneously introduced and integrated to the genome of the host cell, frequently resulting in a tight physical linkage between the different transcription units.

Other types of selectable marker genes which can be used together with the cDNA of the desired protein are based on various transcription units encoding dihydrofolate reductase (dhfr). After introduction of this type of gene into cells lacking endogenous dhfr-activity, preferentially CHO-cells (DUKX-B11, DG-44), it will enable these to grow in media lacking nucleosides. An example of such a medium is Ham's F12 without hypoxanthine, thymidin, and glycine. These dhfr-genes can be introduced together with the FVIII cDNA transcriptional units into CHO-cells of the above type, either linked on the same vector or on different vectors, thus creating dhfr-positive cell lines producing recombinant protein.

If the above cell lines are grown in the presence of the cytotoxic dhfr-inhibitor methotrexate, new cell lines resistant to methotrexate will emerge. These cell lines may produce recombinant protein at an increased rate due to the amplified number of linked dhfr and the desired protein's transcriptional units. When propagating these cell lines in increasing concentrations of methotrexate (1-10000 nM), new cell lines can be obtained which produce the desired protein at very high rate.

The above cell lines producing the desired protein can be grown on a large scale, either in suspension culture or on various solid supports. Examples of these supports are micro carriers based on dextran or collagen matrices, or solid supports in the form of hollow fibres or various ceramic materials. When grown in cell suspension culture or on micro carriers the culture of the above cell lines can be performed either as a bath culture or as a perfusion culture with continuous production of conditioned medium over extended periods of time. Thus, according to the present invention, the above cell lines are well suited for the development of an industrial process for the production of the desired recombinant mutant proteins

Purification and Formulation

The recombinant modified VWF protein, which accumulates in the medium of secreting cells of the above types, can be concentrated and purified by a variety of biochemical and chromatographic methods, including methods utilizing differences in size, charge, hydrophobicity, solubility, specific affinity, etc. between the desired protein and other substances in the cell cultivation medium.

An example of such purification is the adsorption of the recombinant mutant protein to a monoclonal antibody, directed to e.g. a HLEP, preferably human albumin, or directed to the respective coagulation factor, which is immobilised on a solid support. After adsorption of the modified VWF to the support, washing and desorption, the protein can be further purified by a variety of chromatographic techniques based on the above properties. The order of the purification steps is chosen e.g. according to capacity and selectivity of the steps, stability of the support or other aspects. Preferred purification steps e.g. are but are not limited to ion exchange chromatography steps, immune affinity chromatography steps, affinity chromatography steps, hydrophobic interaction chromatography steps, dye chromatography steps, hydroxyapatite chromatography steps, multimodal chromatography steps, and size exclusion chromatography steps.

In order to minimize the theoretical risk of virus contaminations, additional steps may be included in the process that allow effective inactivation or elimination of viruses. Such steps e.g. are heat treatment in the liquid or solid state, treatment with solvents and/or detergents, radiation in the visible or UV spectrum, gamma-radiation or nanofiltration.

The modified polynucleotides (e.g. DNA) of this invention may also be integrated into a transfer vector for use in the human gene therapy.

The various embodiments described herein may be combined with each other. The present invention will be further described in more detail in the following examples thereof. This description of specific embodiments of the invention will be made in conjunction with the appended figures.

The modified VWF as described in this invention can be formulated into pharmaceutical preparations for therapeutic use. The purified protein may be dissolved in conventional physiologically compatible aqueous buffer solutions to which there may be added, optionally, pharmaceutical excipients to provide pharmaceutical preparations.

Such pharmaceutical carriers and excipients as well as suitable pharmaceutical formulations are well known in the art (see for example "Pharmaceutical Formulation Develop-

ment of Peptides and Proteins", Frokjaer et al., Taylor & Francis (2000) or "Handbook of Pharmaceutical Excipients", 3rd edition, Kibbe et al., Pharmaceutical Press (2000)). Standard pharmaceutical formulation techniques are well known to persons skilled in the art (see, e.g., 2005 Physicians' Desk Reference®, Thomson Healthcare: Montvale, N.J., 2004; Remington: The Science and Practice of Pharmacy, 20th ed., Gennaro et al., Eds. Lippincott Williams & Wilkins: Philadelphia, Pa., 2000). In particular, the pharmaceutical composition comprising the polypeptide variant of the invention may be formulated in lyophilized or stable liquid form. The polypeptide variant may be lyophilized by a variety of procedures known in the art. Lyophilized formulations are reconstituted prior to use by the addition of one or more pharmaceutically acceptable diluents such as sterile water for injection or sterile physiological saline solution.

Formulations of the composition are delivered to the individual by any pharmaceutically suitable means of administration. Various delivery systems are known and can be used to administer the composition by any convenient route. Preferentially, the compositions of the invention are administered systemically. For systemic use, insertion proteins of the invention are formulated for parenteral (e.g. intravenous, subcutaneous, intramuscular, intraperitoneal, intracerebral, intrapulmonar, intranasal or transdermal) or enteral (e.g., oral, vaginal or rectal) delivery according to conventional methods. The most preferential routes of administration are intravenous and subcutaneous administration. The formulations can be administered continuously by infusion or by bolus injection. Some formulations encompass slow release systems.

The insertion proteins of the present invention are administered to patients in a therapeutically effective dose, meaning a dose that is sufficient to produce the desired effects, preventing or lessening the severity or spread of the condition or indication being treated without reaching a dose which produces intolerable adverse side effects. The exact dose depends on many factors as e.g. the indication, formulation, mode of administration and has to be determined in preclinical and clinical trials for each respective indication.

The pharmaceutical composition of the invention may be administered alone or in conjunction with other therapeutic agents. These agents may be incorporated as part of the same pharmaceutical. One example of such an agent is the combination of modified VWF with non-modified FVIII or the combination of modified VWF with modified FVIII.

Summary of the nucleotide and amino acid sequences referred to herein:

TABLE 3

SEQ ID NO:	Description
1	nucleotide sequence of DNA encoding SEQ ID NO: 2
2	amino acid sequence of human VWF pre-propolypeptide
3-30	nucleotide sequences of primers, see Examples
31	amino acid sequence of the D' domain of human VWF
32	amino acid sequence of mature human VWF
33	amino acid sequence of the D' domain of mutated human VWF with 15 potentially modified residues
34	amino acid sequence of the D' domain of mutated human VWF with 11 potentially modified residues
35	amino acid sequence of the D' domain of mutated human VWF with 11 potentially modified residues
36	amino acid sequence of human Factor VIII
37	amino acid sequence of a mature single-chain Factor VIII
38	amino acid sequence of human serum albumin

EXAMPLES

Example 1

Generation of Expression Vectors for VWF Mutants

An expression plasmid based on pIRESpuro3 (Clontech) containing a full length VWF cDNA sequence in its multiple cloning site had been generated previously (pVWF-2448). The VWF cDNA sequence contained in this vector is displayed as SEQ ID NO:1, its corresponding protein sequence as SEQ ID NO:2.

For generating such expression vectors, the VWF cDNA may be amplified by polymerase chain reaction (PCR) using primer set VWF+ and VWF− (SEQ ID NO:3 and 4) under standard conditions known to those skilled in the art (and as described e.g. in Current Protocols in Molecular Biology, Ausubel F M et al. (eds.) John Wiley & Sons, Inc.) from a plasmid containing VWF cDNA (as obtainable commercially, e.g. pMT2-VWF from ATCC, No. 67122). The resulting PCR fragment may be digested by restriction endonuclease EcoRI and ligated into expression vector pIRESpuro3 (BD Biosciences, Franklin Lakes, N.J., USA) which had been linearized by EcoRI. The resulting expression plasmid will contain a wild-type cDNA of VWF downstream of the CMV promoter.

In order to introduce mutations in the VWF sequence site directed mutagenesis (QuickChange XL Site Directed Mutagenesis Kit, Stratagene, La Jolla, Calif., USA) was applied on plasmid pVWF-2448 according to the following protocol as suggested by the kit manufacturer. Per mutagenesis reaction 5 μl of 10× reaction buffer, 1 μl of plasmid DNA pVWF-2448 (50 ng), 1 μl (10 pmol/μl) each of the respective two mutagenesis oligonucleotides as outlined in table 4, 1 μl dNTP Mix, 3 μl Quick-Solution, 1 μl Turbo Polymerase (2.5 U/μl) and 37 μl H_2O were mixed and subjected to a polymerase chain reaction with an initial denaturation for 2 min at 95° C., 18 cycles of a) denaturation for 50 sec. at 95° C., b) annealing for 50 sec at 60° C. and c) elongation for 14 min at 68° C., followed by a single terminal elongation phase of 7 min at 68° C. Subsequently 1 μl of Dpnl enzyme from the kit was added and the reaction incubated for another 60 min at 37° C. After that 3 μl of the mutagenesis reaction were transformed into *E. coli*. Clones were isolated, plasmid DNA extracted and the mutations in the VWF sequences were verified by DNA sequencing.

The following table 4 lists the oligonucleotides used for mutagenesis, the respective mutations introduced and the designation of the resulting plasmids with the mutant VWF sequences.

TABLE 4

SEQ ID NO:	Oligonucleotide	Mutagenesis oligonucleotide sequence (5'→3')	VWF mutation (from x to y)	Designation of expression plasmid
5	We4070	GGTGTGTCCCGCTAA CAACCTGCGGGCTG	Asp 779 Asn	pIRES-2462
6	We4071	CAGCCCGCAGGTTGTT AGCGGGACACACC		
7	We4072	GTCCCGCTGACAACCC TCGGGCTGAAGGG	Leu 781 Pro	pIRES-2463
8	We4073	CCCTTCAGCCCGAGGG TTGTCAGCGGGAC		

TABLE 4-continued

SEQ ID NO:	Oligonucleotide	Mutagenesis oligonucleotide sequence (5'→3')	VWF mutation (from x to y)	Designation of expression plasmid
9	We4074	CTGAAGGGCTCGAGTGTG CCAAAACGTGCCAGAAC	Thr 789 Ala	pIRES-2464
10	We4075	GTTCTGGCACGTTTTGGC ACACTCGAGCCCTTCAG		
11	We4076	GTGTACCAAAACGTGCCG GAACTATGACCTGGAGTGC	Gln 793 Arg	pIRES-2465
12	We4077	GCACTCCAGGTCATAGTTC CGGCACGTTTTGGTACAC		
13	We4078	GTACCAAAACGTGCCAGAA GTATGACCTGGAGTGC	Asn 794 Lys	pIRES-2466
14	We4079	GCACTCCAGGTCATACT TCTGGCACGTTTTGGTAC		
15	We4080	CTGGAGTGCATGAGCAGG GGCTGTGTCTCTGGCTG	Met 802 Arg	pIRES-2467
16	We4081	CAGCCAGAGACACAGCC CCTGCTCATGCACTCCAG		
17	We4082	CTGGAGTGCATGAGCAA GGGCTGTGTCTCTGGCTG	Met 802 Lys	pIRES-2468
18	We4083	CAGCCAGAGACACAGCCC TTGCTCATGCACTCCAG		
19	We4084	CATGGTCCGGCATGCCAA CAGATGTGTGGCCCTG	Glu 818 Ala	pIRES-2469
20	We4085	CAGGGCCACACATCTGT TGGCATGCCGGACCATG		
21	We4086	CATGGTCCGGCATAAGAA CAGATGTGTGGCCCTG	Glu 818 Lys	pIRES-2470
22	We4087	CAGGGCCACACATCTGTT CTTATGCCGGACCATG		
23	We4088	GGTCCGGCATGAGAAGA GATGTGTGGCCCTG	Asn 819 Lys	pIRES-2471
24	We4089	CAGGGCCACACATCTCTT CTCATGCCGGACC		
25	We4090	GCTTCCATCAGGGCAAGCA GTATGCCCCTGGAGAAAC	Glu 835 Gln	pIRES-2472
26	We4091	GTTTCTCCAGGGGCATAC TGCTTGCCCTGATGGAAGC		
27	We4092	GGGCAAGGAGTATGCCAAG GGAGAAACAGTGAAGATTGG	Pro 838 Lys	pIRES-2473
28	We4093	CCAATCTTCACTGTTTCTCC CTTGGCATACTCCTTGCCC		
29	We4094	CGGAACCGGAAGTGGAACT GCACAGACCATGTGTG	Asp 853 Asn	pIRES-2474
30	We4095	CACACATGGTCTGTGCAG TTCCACTTCCGGTTCCG		

Using the protocols and plasmids described above and by applying molecular biology techniques known to those skilled in the art (and as described e.g. in Current Protocols in Molecular Biology, ibid) other constructs can be made by the artisan for mutation of other amino acid residues.

Example 2

Transfection of Plasmids and Expression of VWF Mutants in HEK-293 Cells

Expression plasmids were grown up in *E. coli* TOP10 (Invitrogen, Carlsbad, Calif., USA) and purified using standard protocols (Qiagen, Hilden, Germany). HEK-293 cells were transfected using the Lipofectamine 2000 reagent (Invitrogen) and grown up in serum-free medium (Invitrogen 293 Express) in the presence of 4 μg/ml Puromycin. Transfected cell populations were spread through T-flasks into shake flasks from which supernatants were harvested for VWF antigen quantitation and Biacore analysis.

Example 3

Quantitation of VWF Antigen

VWF antigen in culture supernatant was determined by an ELISA whose performance is known to those skilled in the art. Briefly, microplates were incubated with 100 μL per well of the capture antibody (rabbit anti human vWF-IgG, Dako A0082 [Dako, Hamburg, Germany], diluted 1:2000 in buffer A [Sigma C3041, Sigma-Aldrich, Munich, Germany]) overnight at ambient temperature. After washing plates three times with buffer B (Sigma P3563), each well was incubated with 200 μL buffer C (Sigma P3688) for 1.5 hours at ambient temperature (blocking). After another three wash steps with buffer B, serial dilutions of the test sample in buffer B as well as serial dilutions of standard human plasma (ORKL21; 20-0.2 mU/mL; Siemens Healthcare Diagnostics, Marburg, Germany) in buffer B (volumes per well: 100 μL) were incubated for 1.5 hours at ambient temperature. After three wash steps with buffer B, 100 μL of a 1:16000 dilution in buffer B of the detection antibody (rabbit anti human vWF-IgG, Dako P0226, peroxidase labelled) were added to each well and incubated for 1 hour at ambient temperature. After three wash steps with buffer B, 100 μL of substrate solution (OUVF, Siemens Healthcare Diagnostics) were added per well and incubated for 30 minutes at ambient temperature in the dark. Addition of 100 μL undiluted stop dilution (OSFA, Siemens Healthcare Diagnostics) prepared the samples for reading in a suitable microplate reader at 450 nm wavelength. Concentrations of the test samples were then calculated using the standard curve with standard human plasma as reference.

Example 4

Analysis of the Binding of VWF Mutants to FVIII

All binding tests are performed using a Biacore 3000 instrument (GE Healthcare) and CM 3 chips. System buffer and dilution buffer for FVIII products is HBS-P (20 mmol/L Hepes, 100 mmol/L NaCl, 0.005% polysorbate 20, pH 7.3). A monoclonal anti-vWF antibody not interfering with FVIII binding is immobilized by using Biacore amino coupling chemistry. All immobilization, saturation and binding assays are performed at a controlled temperature of 25° C.

Monoclonal anti-vWF antibody is covalently bound to an activated CM 3 chip by NHS and EDC (both from GE Healthcare), a coupling where the antibody is fixed at its aminoterminus to the dextran filaments on the gold surface of the chip. For immobilization the monoclonal antibody is diluted to 10 μg/mL in 10 mM sodium acetate (pH 4.5). The antibody solution is flown over the chip for 8 min at a flowrate of 5 μL/min.

After the immobilization procedure non-coupled dextran filaments are saturated by flowing 1M ethanolamine (pH 8.3) over the chip for 5 min (at a flow rate of 5 μL/min). A reference flow cell is set up by saturating an empty flow cell with ethanolamine by using the same procedure as above.

VWF mutants are immobilized to the covalently coupled anti VWF monoclonal antibody by flowing VWF mutants (in culture supernatant) over the chip until its saturation at a flow rate of 5 μL/min.

For evaluation of the binding of VWF mutants to FVIII, a FVIII preparation is serially diluted in HBS-P buffer, e.g.

to concentrations 0.3125 μg/mL, 0.625 μg/mL, 1.25 μg/mL, 2.5 μg/mL and 5 μg/mL. A sample of each dilution is flown over the chip for 12 min (flow rate 10 μL/min.), followed by a dissociation time of 5 min with HBS-P buffer. After each run the chip is washed with 250 mM $CaCl_2$ for 3 min. to elute FVIII bound to VWF. Thereafter the VWF mutant is stripped by washing with 10 mM glycine (pH 2.1) for 4 min and a the next VWF mutant is bound to the chip as described above.

Binding parameters are calculated using BIAevaluation Software (Biacore, GE Healthcare). The curve fitting methods are based on Langmuir equations. The input data for calculations are the molar masses of the analytes, other parameters like max. RU and slopes are automatically extracted out of the fitted association and dissociation curves. The outputs of Biaevaluation Software are the association rate constants and the dissociation rate constants, from which the affinity constants are calculated.

SEQUENCE LISTING

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ctggcagggg gctgccagaa acgctccttc tcgattattg gggacttcca gaatggcaag 240

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gaaactgagg ctgggtacta caagctgtcc ggtgaggcct atggctttgt ggccaggatc 420

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<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 2

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Ala Arg Cys Ser Leu Phe Gly Ser Asp Phe Val Asn Thr Phe Asp Gly
        35                  40                  45

Ser Met Tyr Ser Phe Ala Gly Tyr Cys Ser Tyr Leu Leu Ala Gly Gly
    50                  55                  60

Cys Gln Lys Arg Ser Phe Ser Ile Ile Gly Asp Phe Gln Asn Gly Lys
65                  70                  75                  80

Arg Val Ser Leu Ser Val Tyr Leu Gly Glu Phe Phe Asp Ile His Leu
                85                  90                  95

Phe Val Asn Gly Thr Val Thr Gln Gly Asp Gln Arg Val Ser Met Pro
            100                 105                 110

Tyr Ala Ser Lys Gly Leu Tyr Leu Glu Thr Glu Ala Gly Tyr Tyr Lys
        115                 120                 125

Leu Ser Gly Glu Ala Tyr Gly Phe Val Ala Arg Ile Asp Gly Ser Gly
    130                 135                 140

Asn Phe Gln Val Leu Leu Ser Asp Arg Tyr Phe Asn Lys Thr Cys Gly
145                 150                 155                 160

Leu Cys Gly Asn Phe Asn Ile Phe Ala Glu Asp Asp Phe Met Thr Gln
                165                 170                 175

Glu Gly Thr Leu Thr Ser Asp Pro Tyr Asp Phe Ala Asn Ser Trp Ala
            180                 185                 190

Leu Ser Ser Gly Glu Gln Trp Cys Glu Arg Ala Ser Pro Pro Ser Ser
        195                 200                 205

Ser Cys Asn Ile Ser Ser Gly Glu Met Gln Lys Gly Leu Trp Glu Gln
    210                 215                 220

Cys Gln Leu Leu Lys Ser Thr Ser Val Phe Ala Arg Cys His Pro Leu
225                 230                 235                 240

Val Asp Pro Glu Pro Phe Val Ala Leu Cys Glu Lys Thr Leu Cys Glu
                245                 250                 255

Cys Ala Gly Gly Leu Glu Cys Ala Cys Pro Ala Leu Leu Glu Tyr Ala
            260                 265                 270

Arg Thr Cys Ala Gln Glu Gly Met Val Leu Tyr Gly Trp Thr Asp His
        275                 280                 285

Ser Ala Cys Ser Pro Val Cys Pro Ala Gly Met Glu Tyr Arg Gln Cys
    290                 295                 300

Val Ser Pro Cys Ala Arg Thr Cys Gln Ser Leu His Ile Asn Glu Met
305                 310                 315                 320

Cys Gln Glu Arg Cys Val Asp Gly Cys Ser Cys Pro Glu Gly Gln Leu
                325                 330                 335

Leu Asp Glu Gly Leu Cys Val Glu Ser Thr Glu Cys Pro Cys Val His
            340                 345                 350

Ser Gly Lys Arg Tyr Pro Pro Gly Thr Ser Leu Ser Arg Asp Cys Asn
        355                 360                 365

Thr Cys Ile Cys Arg Asn Ser Gln Trp Ile Cys Ser Asn Glu Glu Cys
    370                 375                 380

Pro Gly Glu Cys Leu Val Thr Gly Gln Ser His Phe Lys Ser Phe Asp
385                 390                 395                 400

Asn Arg Tyr Phe Thr Phe Ser Gly Ile Cys Gln Tyr Leu Leu Ala Arg
                405                 410                 415

Asp Cys Gln Asp His Ser Phe Ser Ile Val Ile Glu Thr Val Gln Cys
            420                 425                 430
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Ala Asp Asp Arg Asp Ala Val Cys Thr Arg Ser Val Thr Val Arg Leu
        435                 440                 445

Pro Gly Leu His Asn Ser Leu Val Lys Leu Lys His Gly Ala Gly Val
    450                 455                 460

Ala Met Asp Gly Gln Asp Val Gln Leu Pro Leu Leu Lys Gly Asp Leu
465                 470                 475                 480

Arg Ile Gln His Thr Val Thr Ala Ser Val Arg Leu Ser Tyr Gly Glu
                485                 490                 495

Asp Leu Gln Met Asp Trp Asp Gly Arg Gly Arg Leu Leu Val Lys Leu
            500                 505                 510

Ser Pro Val Tyr Ala Gly Lys Thr Cys Gly Leu Cys Gly Asn Tyr Asn
        515                 520                 525

Gly Asn Gln Gly Asp Asp Phe Leu Thr Pro Ser Gly Leu Ala Glu Pro
    530                 535                 540

Arg Val Glu Asp Phe Gly Asn Ala Trp Lys Leu His Gly Asp Cys Gln
545                 550                 555                 560

Asp Leu Gln Lys Gln His Ser Asp Pro Cys Ala Leu Asn Pro Arg Met
                565                 570                 575

Thr Arg Phe Ser Glu Glu Ala Cys Ala Val Leu Thr Ser Pro Thr Phe
            580                 585                 590

Glu Ala Cys His Arg Ala Val Ser Pro Leu Pro Tyr Leu Arg Asn Cys
        595                 600                 605

Arg Tyr Asp Val Cys Ser Cys Ser Asp Gly Arg Glu Cys Leu Cys Gly
    610                 615                 620

Ala Leu Ala Ser Tyr Ala Ala Ala Cys Ala Gly Arg Gly Val Arg Val
625                 630                 635                 640

Ala Trp Arg Glu Pro Gly Arg Cys Glu Leu Asn Cys Pro Lys Gly Gln
                645                 650                 655

Val Tyr Leu Gln Cys Gly Thr Pro Cys Asn Leu Thr Cys Arg Ser Leu
            660                 665                 670

Ser Tyr Pro Asp Glu Glu Cys Asn Glu Ala Cys Leu Glu Gly Cys Phe
        675                 680                 685

Cys Pro Pro Gly Leu Tyr Met Asp Glu Arg Gly Asp Cys Val Pro Lys
    690                 695                 700

Ala Gln Cys Pro Cys Tyr Tyr Asp Gly Glu Ile Phe Gln Pro Glu Asp
705                 710                 715                 720

Ile Phe Ser Asp His His Thr Met Cys Tyr Cys Glu Asp Gly Phe Met
                725                 730                 735

His Cys Thr Met Ser Gly Val Pro Gly Ser Leu Leu Pro Asp Ala Val
            740                 745                 750

Leu Ser Ser Pro Leu Ser His Arg Ser Lys Arg Ser Leu Ser Cys Arg
        755                 760                 765

Pro Pro Met Val Lys Leu Val Cys Pro Ala Asp Asn Leu Arg Ala Glu
    770                 775                 780

Gly Leu Glu Cys Thr Lys Thr Cys Gln Asn Tyr Asp Leu Glu Cys Met
785                 790                 795                 800

Ser Met Gly Cys Val Ser Gly Cys Leu Cys Pro Pro Gly Met Val Arg
                805                 810                 815

His Glu Asn Arg Cys Val Ala Leu Glu Arg Cys Pro Cys Phe His Gln
            820                 825                 830

Gly Lys Glu Tyr Ala Pro Gly Glu Thr Val Lys Ile Gly Cys Asn Thr
        835                 840                 845
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Cys Val Cys Arg Asp Arg Lys Trp Asn Cys Thr Asp His Val Cys Asp
    850                 855                 860

Ala Thr Cys Ser Thr Ile Gly Met Ala His Tyr Leu Thr Phe Asp Gly
865                 870                 875                 880

Leu Lys Tyr Leu Phe Pro Gly Glu Cys Gln Tyr Val Leu Val Gln Asp
                885                 890                 895

Tyr Cys Gly Ser Asn Pro Gly Thr Phe Arg Ile Leu Val Gly Asn Lys
            900                 905                 910

Gly Cys Ser His Pro Ser Val Lys Cys Lys Lys Arg Val Thr Ile Leu
        915                 920                 925

Val Glu Gly Gly Glu Ile Glu Leu Phe Asp Gly Glu Val Asn Val Lys
    930                 935                 940

Arg Pro Met Lys Asp Glu Thr His Phe Glu Val Val Glu Ser Gly Arg
945                 950                 955                 960

Tyr Ile Ile Leu Leu Leu Gly Lys Ala Leu Ser Val Val Trp Asp Arg
                965                 970                 975

His Leu Ser Ile Ser Val Val Leu Lys Gln Thr Tyr Gln Glu Lys Val
            980                 985                 990

Cys Gly Leu Cys Gly Asn Phe Asp  Gly Ile Gln Asn Asn  Asp Leu Thr
        995                 1000                1005

Ser Ser  Asn Leu Gln Val Glu  Glu Asp Pro Val Asp  Phe Gly Asn
    1010                1015                1020

Ser Trp  Lys Val Ser Ser Gln  Cys Ala Asp Thr Arg  Lys Val Pro
    1025                1030                1035

Leu Asp  Ser Ser Pro Ala Thr  Cys His Asn Asn Ile  Met Lys Gln
    1040                1045                1050

Thr Met  Val Asp Ser Ser Cys  Arg Ile Leu Thr Ser  Asp Val Phe
    1055                1060                1065

Gln Asp  Cys Asn Lys Leu Val  Asp Pro Glu Pro Tyr  Leu Asp Val
    1070                1075                1080

Cys Ile  Tyr Asp Thr Cys Ser  Cys Glu Ser Ile Gly  Asp Cys Ala
    1085                1090                1095

Cys Phe  Cys Asp Thr Ile Ala  Ala Tyr Ala His Val  Cys Ala Gln
    1100                1105                1110

His Gly  Lys Val Val Thr Trp  Arg Thr Ala Thr Leu  Cys Pro Gln
    1115                1120                1125

Ser Cys  Glu Glu Arg Asn Leu  Arg Glu Asn Gly Tyr  Glu Cys Glu
    1130                1135                1140

Trp Arg  Tyr Asn Ser Cys Ala  Pro Ala Cys Gln Val  Thr Cys Gln
    1145                1150                1155

His Pro  Glu Pro Leu Ala Cys  Pro Val Gln Cys Val  Glu Gly Cys
    1160                1165                1170

His Ala  His Cys Pro Pro Gly  Lys Ile Leu Asp Glu  Leu Leu Gln
    1175                1180                1185

Thr Cys  Val Asp Pro Glu Asp  Cys Pro Val Cys Glu  Val Ala Gly
    1190                1195                1200

Arg Arg  Phe Ala Ser Gly Lys  Lys Val Thr Leu Asn  Pro Ser Asp
    1205                1210                1215

Pro Glu  His Cys Gln Ile Cys  His Cys Asp Val Val  Asn Leu Thr
    1220                1225                1230

Cys Glu  Ala Cys Gln Glu Pro  Gly Gly Leu Val Val  Pro Pro Thr
    1235                1240                1245

Asp Ala  Pro Val Ser Pro Thr  Thr Leu Tyr Val Glu  Asp Ile Ser
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Glu Pro  Pro Leu His Asp Phe  Tyr Cys Ser Arg Leu  Leu Asp Leu
    1265                1270                1275

Val Phe  Leu Leu Asp Gly Ser  Ser Arg Leu Ser Glu  Ala Glu Phe
    1280                1285                1290

Glu Val  Leu Lys Ala Phe Val  Val Asp Met Met Glu  Arg Leu Arg
    1295                1300                1305

Ile Ser  Gln Lys Trp Val Arg  Val Ala Val Val Glu  Tyr His Asp
    1310                1315                1320

Gly Ser  His Ala Tyr Ile Gly  Leu Lys Asp Arg Lys  Arg Pro Ser
    1325                1330                1335

Glu Leu  Arg Arg Ile Ala Ser  Gln Val Lys Tyr Ala  Gly Ser Gln
    1340                1345                1350

Val Ala  Ser Thr Ser Glu Val  Leu Lys Tyr Thr Leu  Phe Gln Ile
    1355                1360                1365

Phe Ser  Lys Ile Asp Arg Pro  Glu Ala Ser Arg Ile  Thr Leu Leu
    1370                1375                1380

Leu Met  Ala Ser Gln Glu Pro  Gln Arg Met Ser Arg  Asn Phe Val
    1385                1390                1395

Arg Tyr  Val Gln Gly Leu Lys  Lys Lys Lys Val Ile  Val Ile Pro
    1400                1405                1410

Val Gly  Ile Gly Pro His Ala  Asn Leu Lys Gln Ile  Arg Leu Ile
    1415                1420                1425

Glu Lys  Gln Ala Pro Glu Asn  Lys Ala Phe Val Leu  Ser Ser Val
    1430                1435                1440

Asp Glu  Leu Glu Gln Gln Arg  Asp Glu Ile Val Ser  Tyr Leu Cys
    1445                1450                1455

Asp Leu  Ala Pro Glu Ala Pro  Pro Pro Thr Leu Pro  Pro Asp Met
    1460                1465                1470

Ala Gln  Val Thr Val Gly Pro  Gly Leu Leu Gly Val  Ser Thr Leu
    1475                1480                1485

Gly Pro  Lys Arg Asn Ser Met  Val Leu Asp Val Ala  Phe Val Leu
    1490                1495                1500

Glu Gly  Ser Asp Lys Ile Gly  Glu Ala Asp Phe Asn  Arg Ser Lys
    1505                1510                1515

Glu Phe  Met Glu Glu Val Ile  Gln Arg Met Asp Val  Gly Gln Asp
    1520                1525                1530

Ser Ile  His Val Thr Val Leu  Gln Tyr Ser Tyr Met  Val Thr Val
    1535                1540                1545

Glu Tyr  Pro Phe Ser Glu Ala  Gln Ser Lys Gly Asp  Ile Leu Gln
    1550                1555                1560

Arg Val  Arg Glu Ile Arg Tyr  Gln Gly Gly Asn Arg  Thr Asn Thr
    1565                1570                1575

Gly Leu  Ala Leu Arg Tyr Leu  Ser Asp His Ser Phe  Leu Val Ser
    1580                1585                1590

Gln Gly  Asp Arg Glu Gln Ala  Pro Asn Leu Val Tyr  Met Val Thr
    1595                1600                1605

Gly Asn  Pro Ala Ser Asp Glu  Ile Lys Arg Leu Pro  Gly Asp Ile
    1610                1615                1620

Gln Val  Val Pro Ile Gly Val  Gly Pro Asn Ala Asn  Val Gln Glu
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Leu Glu  Arg Ile Gly Trp Pro  Asn Ala Pro Ile Leu  Ile Gln Asp
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Phe Glu  Thr Leu Pro Arg Glu  Ala Pro Asp Leu Val  Leu Gln Arg
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Cys Cys  Ser Gly Glu Gly Leu  Gln Ile Pro Thr Leu  Ser Pro Ala
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Pro Asp  Cys Ser Gln Pro Leu  Asp Val Ile Leu Leu  Leu Asp Gly
    1685                 1690                 1695

Ser Ser  Ser Phe Pro Ala Ser  Tyr Phe Asp Glu Met  Lys Ser Phe
    1700                 1705                 1710

Ala Lys  Ala Phe Ile Ser Lys  Ala Asn Ile Gly Pro  Arg Leu Thr
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Gln Val  Ser Val Leu Gln Tyr  Gly Ser Ile Thr Thr  Ile Asp Val
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Pro Trp  Asn Val Val Pro Glu  Lys Ala His Leu Leu  Ser Leu Val
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Asp Val  Met Gln Arg Glu Gly  Gly Pro Ser Gln Ile  Gly Asp Ala
    1760                 1765                 1770

Leu Gly  Phe Ala Val Arg Tyr  Leu Thr Ser Glu Met  His Gly Ala
    1775                 1780                 1785

Arg Pro  Gly Ala Ser Lys Ala  Val Val Ile Leu Val  Thr Asp Val
    1790                 1795                 1800

Ser Val  Asp Ser Val Asp Ala  Ala Ala Asp Ala Ala  Arg Ser Asn
    1805                 1810                 1815

Arg Val  Thr Val Phe Pro Ile  Gly Ile Gly Asp Arg  Tyr Asp Ala
    1820                 1825                 1830

Ala Gln  Leu Arg Ile Leu Ala  Gly Pro Ala Gly Asp  Ser Asn Val
    1835                 1840                 1845

Val Lys  Leu Gln Arg Ile Glu  Asp Leu Pro Thr Met  Val Thr Leu
    1850                 1855                 1860

Gly Asn  Ser Phe Leu His Lys  Leu Cys Ser Gly Phe  Val Arg Ile
    1865                 1870                 1875

Cys Met  Asp Glu Asp Gly Asn  Glu Lys Arg Pro Gly  Asp Val Trp
    1880                 1885                 1890

Thr Leu  Pro Asp Gln Cys His  Thr Val Thr Cys Gln  Pro Asp Gly
    1895                 1900                 1905

Gln Thr  Leu Leu Lys Ser His  Arg Val Asn Cys Asp  Arg Gly Leu
    1910                 1915                 1920

Arg Pro  Ser Cys Pro Asn Ser  Gln Ser Pro Val Lys  Val Glu Glu
    1925                 1930                 1935

Thr Cys  Gly Cys Arg Trp Thr  Cys Pro Cys Val Cys  Thr Gly Ser
    1940                 1945                 1950

Ser Thr  Arg His Ile Val Thr  Phe Asp Gly Gln Asn  Phe Lys Leu
    1955                 1960                 1965

Thr Gly  Ser Cys Ser Tyr Val  Leu Phe Gln Asn Lys  Glu Gln Asp
    1970                 1975                 1980

Leu Glu  Val Ile Leu His Asn  Gly Ala Cys Ser Pro  Gly Ala Arg
    1985                 1990                 1995

Gln Gly  Cys Met Lys Ser Ile  Glu Val Lys His Ser  Ala Leu Ser
    2000                 2005                 2010

Val Glu  Leu His Ser Asp Met  Glu Val Thr Val Asn  Gly Arg Leu
    2015                 2020                 2025

Val Ser  Val Pro Tyr Val Gly  Gly Asn Met Glu Val  Asn Val Tyr
    2030                 2035                 2040
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Gly Ala  Ile Met His Glu Val  Arg Phe Asn His Leu  Gly His Ile
    2045                2050                2055

Phe Thr  Phe Thr Pro Gln Asn  Asn Glu Phe Gln Leu  Gln Leu Ser
    2060                2065                2070

Pro Lys  Thr Phe Ala Ser Lys  Thr Tyr Gly Leu Cys  Gly Ile Cys
    2075                2080                2085

Asp Glu  Asn Gly Ala Asn Asp  Phe Met Leu Arg Asp  Gly Thr Val
    2090                2095                2100

Thr Thr  Asp Trp Lys Thr Leu  Val Gln Glu Trp Thr  Val Gln Arg
    2105                2110                2115

Pro Gly  Gln Thr Cys Gln Pro  Ile Leu Glu Glu Gln  Cys Leu Val
    2120                2125                2130

Pro Asp  Ser Ser His Cys Gln  Val Leu Leu Leu Pro  Leu Phe Ala
    2135                2140                2145

Glu Cys  His Lys Val Leu Ala  Pro Ala Thr Phe Tyr  Ala Ile Cys
    2150                2155                2160

Gln Gln  Asp Ser Cys His Gln  Glu Gln Val Cys Glu  Val Ile Ala
    2165                2170                2175

Ser Tyr  Ala His Leu Cys Arg  Thr Asn Gly Val Cys  Val Asp Trp
    2180                2185                2190

Arg Thr  Pro Asp Phe Cys Ala  Met Ser Cys Pro Pro  Ser Leu Val
    2195                2200                2205

Tyr Asn  His Cys Glu His Gly  Cys Pro Arg His Cys  Asp Gly Asn
    2210                2215                2220

Val Ser  Ser Cys Gly Asp His  Pro Ser Glu Gly Cys  Phe Cys Pro
    2225                2230                2235

Pro Asp  Lys Val Met Leu Glu  Gly Ser Cys Val Pro  Glu Glu Ala
    2240                2245                2250

Cys Thr  Gln Cys Ile Gly Glu  Asp Gly Val Gln His  Gln Phe Leu
    2255                2260                2265

Glu Ala  Trp Val Pro Asp His  Gln Pro Cys Gln Ile  Cys Thr Cys
    2270                2275                2280

Leu Ser  Gly Arg Lys Val Asn  Cys Thr Thr Gln Pro  Cys Pro Thr
    2285                2290                2295

Ala Lys  Ala Pro Thr Cys Gly  Leu Cys Glu Val Ala  Arg Leu Arg
    2300                2305                2310

Gln Asn  Ala Asp Gln Cys Cys  Pro Glu Tyr Glu Cys  Val Cys Asp
    2315                2320                2325

Pro Val  Ser Cys Asp Leu Pro  Pro Val Pro His Cys  Glu Arg Gly
    2330                2335                2340

Leu Gln  Pro Thr Leu Thr Asn  Pro Gly Glu Cys Arg  Pro Asn Phe
    2345                2350                2355

Thr Cys  Ala Cys Arg Lys Glu  Glu Cys Lys Arg Val  Ser Pro Pro
    2360                2365                2370

Ser Cys  Pro Pro His Arg Leu  Pro Thr Leu Arg Lys  Thr Gln Cys
    2375                2380                2385

Cys Asp  Glu Tyr Glu Cys Ala  Cys Asn Cys Val Asn  Ser Thr Val
    2390                2395                2400

Ser Cys  Pro Leu Gly Tyr Leu  Ala Ser Thr Ala Thr  Asn Asp Cys
    2405                2410                2415

Gly Cys  Thr Thr Thr Thr Cys  Leu Pro Asp Lys Val  Cys Val His
    2420                2425                2430

Arg Ser  Thr Ile Tyr Pro Val  Gly Gln Phe Trp Glu  Glu Gly Cys
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    2435                2440                2445

Asp Val  Cys Thr Cys Thr Asp  Met Glu Asp Ala Val  Met Gly Leu
    2450                2455                2460

Arg Val  Ala Gln Cys Ser Gln  Lys Pro Cys Glu Asp  Ser Cys Arg
    2465                2470                2475

Ser Gly  Phe Thr Tyr Val Leu  His Glu Gly Glu Cys  Cys Gly Arg
    2480                2485                2490

Cys Leu  Pro Ser Ala Cys Glu  Val Val Thr Gly Ser  Pro Arg Gly
    2495                2500                2505

Asp Ser  Gln Ser Ser Trp Lys  Ser Val Gly Ser Gln  Trp Ala Ser
    2510                2515                2520

Pro Glu  Asn Pro Cys Leu Ile  Asn Glu Cys Val Arg  Val Lys Glu
    2525                2530                2535

Glu Val  Phe Ile Gln Gln Arg  Asn Val Ser Cys Pro  Gln Leu Glu
    2540                2545                2550

Val Pro  Val Cys Pro Ser Gly  Phe Gln Leu Ser Cys  Lys Thr Ser
    2555                2560                2565

Ala Cys  Cys Pro Ser Cys Arg  Cys Glu Arg Met Glu  Ala Cys Met
    2570                2575                2580

Leu Asn  Gly Thr Val Ile Gly  Pro Gly Lys Thr Val  Met Ile Asp
    2585                2590                2595

Val Cys  Thr Thr Cys Arg Cys  Met Val Gln Val Gly  Val Ile Ser
    2600                2605                2610

Gly Phe  Lys Leu Glu Cys Arg  Lys Thr Thr Cys Asn  Pro Cys Pro
    2615                2620                2625

Leu Gly  Tyr Lys Glu Glu Asn  Asn Thr Gly Glu Cys  Cys Gly Arg
    2630                2635                2640

Cys Leu  Pro Thr Ala Cys Thr  Ile Gln Leu Arg Gly  Gly Gln Ile
    2645                2650                2655

Met Thr  Leu Lys Arg Asp Glu  Thr Leu Gln Asp Gly  Cys Asp Thr
    2660                2665                2670

His Phe  Cys Lys Val Asn Glu  Arg Gly Glu Tyr Phe  Trp Glu Lys
    2675                2680                2685

Arg Val  Thr Gly Cys Pro Pro  Phe Asp Glu His Lys  Cys Leu Ala
    2690                2695                2700

Glu Gly  Gly Lys Ile Met Lys  Ile Pro Gly Thr Cys  Cys Asp Thr
    2705                2710                2715

Cys Glu  Glu Pro Glu Cys Asn  Asp Ile Thr Ala Arg  Leu Gln Tyr
    2720                2725                2730

Val Lys  Val Gly Ser Cys Lys  Ser Glu Val Glu Val  Asp Ile His
    2735                2740                2745

Tyr Cys  Gln Gly Lys Cys Ala  Ser Lys Ala Met Tyr  Ser Ile Asp
    2750                2755                2760

Ile Asn  Asp Val Gln Asp Gln  Cys Ser Cys Cys Ser  Pro Thr Arg
    2765                2770                2775

Thr Glu  Pro Met Gln Val Ala  Leu His Cys Thr Asn  Gly Ser Val
    2780                2785                2790

Val Tyr  His Glu Val Leu Asn  Ala Met Glu Cys Lys  Cys Ser Pro
    2795                2800                2805

Arg Lys  Cys Ser Lys
    2810
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<210> SEQ ID NO 3

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<211> LENGTH: 30
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer VWF+

<400> SEQUENCE: 3

ttcgaattcc cgcagccctc atttgcaggg 30

<210> SEQ ID NO 4
<211> LENGTH: 31
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer VWF-

<400> SEQUENCE: 4

tccgaattcc ggcagcagca ggcacccatg c 31

<210> SEQ ID NO 5
<211> LENGTH: 29
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer We4070

<400> SEQUENCE: 5

ggtgtgtccc gctaacaacc tgcgggctg 29

<210> SEQ ID NO 6
<211> LENGTH: 29
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer We4071

<400> SEQUENCE: 6

cagcccgcag gttgttagcg ggacacacc 29

<210> SEQ ID NO 7
<211> LENGTH: 29
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Pirmer We4072

<400> SEQUENCE: 7

gtcccgctga caaccctcgg gctgaaggg 29

<210> SEQ ID NO 8
<211> LENGTH: 29
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer We4073

<400> SEQUENCE: 8

cccttcagcc cgagggttgt cagcgggac 29

<210> SEQ ID NO 9
<211> LENGTH: 35
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer We4074

<400> SEQUENCE: 9

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ctgaagggct cgagtgtgcc aaaacgtgcc agaac 35

<210> SEQ ID NO 10
<211> LENGTH: 35
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer We4075

<400> SEQUENCE: 10

gttctggcac gttttggcac actcgagccc ttcag 35

<210> SEQ ID NO 11
<211> LENGTH: 37
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer We4076

<400> SEQUENCE: 11

gtgtaccaaa acgtgccgga actatgacct ggagtgc 37

<210> SEQ ID NO 12
<211> LENGTH: 37
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer We4077

<400> SEQUENCE: 12

gcactccagg tcatagttcc ggcacgtttt ggtacac 37

<210> SEQ ID NO 13
<211> LENGTH: 35
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer We4078

<400> SEQUENCE: 13

gtaccaaaac gtgccagaag tatgacctgg agtgc 35

<210> SEQ ID NO 14
<211> LENGTH: 35
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: We4079

<400> SEQUENCE: 14

gcactccagg tcatacttct ggcacgtttt ggtac 35

<210> SEQ ID NO 15
<211> LENGTH: 35
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer We4080

<400> SEQUENCE: 15

ctggagtgca tgagcagggg ctgtgtctct ggctg 35

<210> SEQ ID NO 16
<211> LENGTH: 35
<212> TYPE: DNA

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<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer We4081

<400> SEQUENCE: 16

cagccagaga cacagcccct gctcatgcac tccag 35

<210> SEQ ID NO 17
<211> LENGTH: 35
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer We4082

<400> SEQUENCE: 17

ctggagtgca tgagcaaggg ctgtgtctct ggctg 35

<210> SEQ ID NO 18
<211> LENGTH: 35
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer We4083

<400> SEQUENCE: 18

cagccagaga cacagccctt gctcatgcac tccag 35

<210> SEQ ID NO 19
<211> LENGTH: 34
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer We4084

<400> SEQUENCE: 19

catggtccgg catgccaaca gatgtgtggc cctg 34

<210> SEQ ID NO 20
<211> LENGTH: 34
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer We4085

<400> SEQUENCE: 20

cagggccaca catctgttgg catgccggac catg 34

<210> SEQ ID NO 21
<211> LENGTH: 34
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer We4086

<400> SEQUENCE: 21

catggtccgg cataagaaca gatgtgtggc cctg 34

<210> SEQ ID NO 22
<211> LENGTH: 34
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer We4087

<400> SEQUENCE: 22

cagggccaca catctgttct tatgccggac catg 34

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<210> SEQ ID NO 23
<211> LENGTH: 31
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer We4088

<400> SEQUENCE: 23

ggtccggcat gagaagagat gtgtggccct g 31

<210> SEQ ID NO 24
<211> LENGTH: 31
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer We4089

<400> SEQUENCE: 24

cagggccaca catctcttct catgccggac c 31

<210> SEQ ID NO 25
<211> LENGTH: 37
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer We4090

<400> SEQUENCE: 25

gcttccatca gggcaagcag tatgcccctg gagaaac 37

<210> SEQ ID NO 26
<211> LENGTH: 37
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer We4091

<400> SEQUENCE: 26

gtttctccag gggcatactg cttgccctga tggaagc 37

<210> SEQ ID NO 27
<211> LENGTH: 39
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer We4092

<400> SEQUENCE: 27

gggcaaggag tatgccaagg gagaaacagt gaagattgg 39

<210> SEQ ID NO 28
<211> LENGTH: 39
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer We4093

<400> SEQUENCE: 28

ccaatcttca ctgtttctcc cttggcatac tccttgccc 39

<210> SEQ ID NO 29
<211> LENGTH: 35
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:

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<223> OTHER INFORMATION: Primer We4094

<400> SEQUENCE: 29

cggaaccgga agtggaactg cacagaccat gtgtg 35

<210> SEQ ID NO 30
<211> LENGTH: 35
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer We4095

<400> SEQUENCE: 30

cacacatggt ctgtgcagtt ccacttccgg ttccg 35

<210> SEQ ID NO 31
<211> LENGTH: 102
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 31

Ser Leu Ser Cys Arg Pro Pro Met Val Lys Leu Val Cys Pro Ala Asp
1 5 10 15

Asn Leu Arg Ala Glu Gly Leu Glu Cys Thr Lys Thr Cys Gln Asn Tyr
20 25 30

Asp Leu Glu Cys Met Ser Met Gly Cys Val Ser Gly Cys Leu Cys Pro
35 40 45

Pro Gly Met Val Arg His Glu Asn Arg Cys Val Ala Leu Glu Arg Cys
50 55 60

Pro Cys Phe His Gln Gly Lys Glu Tyr Ala Pro Gly Glu Thr Val Lys
65 70 75 80

Ile Gly Cys Asn Thr Cys Val Cys Arg Asp Arg Lys Trp Asn Cys Thr
85 90 95

Asp His Val Cys Asp Ala
100

<210> SEQ ID NO 32
<211> LENGTH: 2050
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 32

Ser Leu Ser Cys Arg Pro Pro Met Val Lys Leu Val Cys Pro Ala Asp
1 5 10 15

Asn Leu Arg Ala Glu Gly Leu Glu Cys Thr Lys Thr Cys Gln Asn Tyr
20 25 30

Asp Leu Glu Cys Met Ser Met Gly Cys Val Ser Gly Cys Leu Cys Pro
35 40 45

Pro Gly Met Val Arg His Glu Asn Arg Cys Val Ala Leu Glu Arg Cys
50 55 60

Pro Cys Phe His Gln Gly Lys Glu Tyr Ala Pro Gly Glu Thr Val Lys
65 70 75 80

Ile Gly Cys Asn Thr Cys Val Cys Arg Asp Arg Lys Trp Asn Cys Thr
85 90 95

Asp His Val Cys Asp Ala Thr Cys Ser Thr Ile Gly Met Ala His Tyr
100 105 110

Leu Thr Phe Asp Gly Leu Lys Tyr Leu Phe Pro Gly Glu Cys Gln Tyr
115 120 125

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Val Leu Val Gln Asp Tyr Cys Gly Ser Asn Pro Gly Thr Phe Arg Ile
    130                 135                 140

Leu Val Gly Asn Lys Gly Cys Ser His Pro Ser Val Lys Cys Lys Lys
145                 150                 155                 160

Arg Val Thr Ile Leu Val Glu Gly Gly Glu Ile Glu Leu Phe Asp Gly
                165                 170                 175

Glu Val Asn Val Lys Arg Pro Met Lys Asp Glu Thr His Phe Glu Val
            180                 185                 190

Val Glu Ser Gly Arg Tyr Ile Ile Leu Leu Leu Gly Lys Ala Leu Ser
        195                 200                 205

Val Val Trp Asp Arg His Leu Ser Ile Ser Val Val Leu Lys Gln Thr
    210                 215                 220

Tyr Gln Glu Lys Val Cys Gly Leu Cys Gly Asn Phe Asp Gly Ile Gln
225                 230                 235                 240

Asn Asn Asp Leu Thr Ser Ser Asn Leu Gln Val Glu Glu Asp Pro Val
                245                 250                 255

Asp Phe Gly Asn Ser Trp Lys Val Ser Ser Gln Cys Ala Asp Thr Arg
            260                 265                 270

Lys Val Pro Leu Asp Ser Ser Pro Ala Thr Cys His Asn Asn Ile Met
        275                 280                 285

Lys Gln Thr Met Val Asp Ser Ser Cys Arg Ile Leu Thr Ser Asp Val
    290                 295                 300

Phe Gln Asp Cys Asn Lys Leu Val Asp Pro Glu Pro Tyr Leu Asp Val
305                 310                 315                 320

Cys Ile Tyr Asp Thr Cys Ser Cys Glu Ser Ile Gly Asp Cys Ala Cys
                325                 330                 335

Phe Cys Asp Thr Ile Ala Ala Tyr Ala His Val Cys Ala Gln His Gly
            340                 345                 350

Lys Val Val Thr Trp Arg Thr Ala Thr Leu Cys Pro Gln Ser Cys Glu
        355                 360                 365

Glu Arg Asn Leu Arg Glu Asn Gly Tyr Glu Cys Glu Trp Arg Tyr Asn
    370                 375                 380

Ser Cys Ala Pro Ala Cys Gln Val Thr Cys Gln His Pro Glu Pro Leu
385                 390                 395                 400

Ala Cys Pro Val Gln Cys Val Glu Gly Cys His Ala His Cys Pro Pro
                405                 410                 415

Gly Lys Ile Leu Asp Glu Leu Leu Gln Thr Cys Val Asp Pro Glu Asp
            420                 425                 430

Cys Pro Val Cys Glu Val Ala Gly Arg Arg Phe Ala Ser Gly Lys Lys
        435                 440                 445

Val Thr Leu Asn Pro Ser Asp Pro Glu His Cys Gln Ile Cys His Cys
    450                 455                 460

Asp Val Val Asn Leu Thr Cys Glu Ala Cys Gln Glu Pro Gly Gly Leu
465                 470                 475                 480

Val Val Pro Pro Thr Asp Ala Pro Val Ser Pro Thr Thr Leu Tyr Val
                485                 490                 495

Glu Asp Ile Ser Glu Pro Pro Leu His Asp Phe Tyr Cys Ser Arg Leu
            500                 505                 510

Leu Asp Leu Val Phe Leu Leu Asp Gly Ser Ser Arg Leu Ser Glu Ala
        515                 520                 525

Glu Phe Glu Val Leu Lys Ala Phe Val Val Asp Met Met Glu Arg Leu
    530                 535                 540

Arg Ile Ser Gln Lys Trp Val Arg Val Ala Val Val Glu Tyr His Asp
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545                 550                 555                 560

Gly Ser His Ala Tyr Ile Gly Leu Lys Asp Arg Lys Arg Pro Ser Glu
                565                 570                 575

Leu Arg Arg Ile Ala Ser Gln Val Lys Tyr Ala Gly Ser Gln Val Ala
            580                 585                 590

Ser Thr Ser Glu Val Leu Lys Tyr Thr Leu Phe Gln Ile Phe Ser Lys
        595                 600                 605

Ile Asp Arg Pro Glu Ala Ser Arg Ile Thr Leu Leu Leu Met Ala Ser
    610                 615                 620

Gln Glu Pro Gln Arg Met Ser Arg Asn Phe Val Arg Tyr Val Gln Gly
625                 630                 635                 640

Leu Lys Lys Lys Lys Val Ile Val Ile Pro Val Gly Ile Gly Pro His
                645                 650                 655

Ala Asn Leu Lys Gln Ile Arg Leu Ile Glu Lys Gln Ala Pro Glu Asn
            660                 665                 670

Lys Ala Phe Val Leu Ser Ser Val Asp Glu Leu Glu Gln Gln Arg Asp
        675                 680                 685

Glu Ile Val Ser Tyr Leu Cys Asp Leu Ala Pro Glu Ala Pro Pro Pro
    690                 695                 700

Thr Leu Pro Pro Asp Met Ala Gln Val Thr Val Gly Pro Gly Leu Leu
705                 710                 715                 720

Gly Val Ser Thr Leu Gly Pro Lys Arg Asn Ser Met Val Leu Asp Val
                725                 730                 735

Ala Phe Val Leu Glu Gly Ser Asp Lys Ile Gly Glu Ala Asp Phe Asn
            740                 745                 750

Arg Ser Lys Glu Phe Met Glu Glu Val Ile Gln Arg Met Asp Val Gly
        755                 760                 765

Gln Asp Ser Ile His Val Thr Val Leu Gln Tyr Ser Tyr Met Val Thr
    770                 775                 780

Val Glu Tyr Pro Phe Ser Glu Ala Gln Ser Lys Gly Asp Ile Leu Gln
785                 790                 795                 800

Arg Val Arg Glu Ile Arg Tyr Gln Gly Gly Asn Arg Thr Asn Thr Gly
                805                 810                 815

Leu Ala Leu Arg Tyr Leu Ser Asp His Ser Phe Leu Val Ser Gln Gly
            820                 825                 830

Asp Arg Glu Gln Ala Pro Asn Leu Val Tyr Met Val Thr Gly Asn Pro
        835                 840                 845

Ala Ser Asp Glu Ile Lys Arg Leu Pro Gly Asp Ile Gln Val Val Pro
    850                 855                 860

Ile Gly Val Gly Pro Asn Ala Asn Val Gln Glu Leu Glu Arg Ile Gly
865                 870                 875                 880

Trp Pro Asn Ala Pro Ile Leu Ile Gln Asp Phe Glu Thr Leu Pro Arg
                885                 890                 895

Glu Ala Pro Asp Leu Val Leu Gln Arg Cys Cys Ser Gly Glu Gly Leu
            900                 905                 910

Gln Ile Pro Thr Leu Ser Pro Ala Pro Asp Cys Ser Gln Pro Leu Asp
        915                 920                 925

Val Ile Leu Leu Leu Asp Gly Ser Ser Ser Phe Pro Ala Ser Tyr Phe
    930                 935                 940

Asp Glu Met Lys Ser Phe Ala Lys Ala Phe Ile Ser Lys Ala Asn Ile
945                 950                 955                 960

Gly Pro Arg Leu Thr Gln Val Ser Val Leu Gln Tyr Gly Ser Ile Thr
                965                 970                 975
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```
Thr Ile Asp Val Pro Trp Asn Val Val Pro Glu Lys Ala His Leu Leu
            980                 985                 990

Ser Leu Val Asp Val Met Gln Arg  Glu Gly Gly Pro Ser  Gln Ile Gly
        995                1000                1005

Asp Ala  Leu Gly Phe Ala Val  Arg Tyr Leu Thr Ser  Glu Met His
    1010                1015                1020

Gly Ala  Arg Pro Gly Ala Ser  Lys Ala Val Val Ile  Leu Val Thr
    1025                1030                1035

Asp Val  Ser Val Asp Ser Val  Asp Ala Ala Ala Asp  Ala Ala Arg
    1040                1045                1050

Ser Asn  Arg Val Thr Val Phe  Pro Ile Gly Ile Gly  Asp Arg Tyr
    1055                1060                1065

Asp Ala  Ala Gln Leu Arg Ile  Leu Ala Gly Pro Ala  Gly Asp Ser
    1070                1075                1080

Asn Val  Val Lys Leu Gln Arg  Ile Glu Asp Leu Pro  Thr Met Val
    1085                1090                1095

Thr Leu  Gly Asn Ser Phe Leu  His Lys Leu Cys Ser  Gly Phe Val
    1100                1105                1110

Arg Ile  Cys Met Asp Glu Asp  Gly Asn Glu Lys Arg  Pro Gly Asp
    1115                1120                1125

Val Trp  Thr Leu Pro Asp Gln  Cys His Thr Val Thr  Cys Gln Pro
    1130                1135                1140

Asp Gly  Gln Thr Leu Leu Lys  Ser His Arg Val Asn  Cys Asp Arg
    1145                1150                1155

Gly Leu  Arg Pro Ser Cys Pro  Asn Ser Gln Ser Pro  Val Lys Val
    1160                1165                1170

Glu Glu  Thr Cys Gly Cys Arg  Trp Thr Cys Pro Cys  Val Cys Thr
    1175                1180                1185

Gly Ser  Ser Thr Arg His Ile  Val Thr Phe Asp Gly  Gln Asn Phe
    1190                1195                1200

Lys Leu  Thr Gly Ser Cys Ser  Tyr Val Leu Phe Gln  Asn Lys Glu
    1205                1210                1215

Gln Asp  Leu Glu Val Ile Leu  His Asn Gly Ala Cys  Ser Pro Gly
    1220                1225                1230

Ala Arg  Gln Gly Cys Met Lys  Ser Ile Glu Val Lys  His Ser Ala
    1235                1240                1245

Leu Ser  Val Glu Leu His Ser  Asp Met Glu Val Thr  Val Asn Gly
    1250                1255                1260

Arg Leu  Val Ser Val Pro Tyr  Val Gly Gly Asn Met  Glu Val Asn
    1265                1270                1275

Val Tyr  Gly Ala Ile Met His  Glu Val Arg Phe Asn  His Leu Gly
    1280                1285                1290

His Ile  Phe Thr Phe Thr Pro  Gln Asn Asn Glu Phe  Gln Leu Gln
    1295                1300                1305

Leu Ser  Pro Lys Thr Phe Ala  Ser Lys Thr Tyr Gly  Leu Cys Gly
    1310                1315                1320

Ile Cys  Asp Glu Asn Gly Ala  Asn Asp Phe Met Leu  Arg Asp Gly
    1325                1330                1335

Thr Val  Thr Thr Asp Trp Lys  Thr Leu Val Gln Glu  Trp Thr Val
    1340                1345                1350

Gln Arg  Pro Gly Gln Thr Cys  Gln Pro Ile Leu Glu  Glu Gln Cys
    1355                1360                1365
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Leu Val  Pro Asp Ser Ser His  Cys Gln Val Leu Leu  Leu Pro Leu
    1370                1375                1380

Phe Ala  Glu Cys His Lys Val  Leu Ala Pro Ala Thr  Phe Tyr Ala
    1385                1390                1395

Ile Cys  Gln Gln Asp Ser Cys  His Gln Glu Gln Val  Cys Glu Val
    1400                1405                1410

Ile Ala  Ser Tyr Ala His Leu  Cys Arg Thr Asn Gly  Val Cys Val
    1415                1420                1425

Asp Trp  Arg Thr Pro Asp Phe  Cys Ala Met Ser Cys  Pro Pro Ser
    1430                1435                1440

Leu Val  Tyr Asn His Cys Glu  His Gly Cys Pro Arg  His Cys Asp
    1445                1450                1455

Gly Asn  Val Ser Ser Cys Gly  Asp His Pro Ser Glu  Gly Cys Phe
    1460                1465                1470

Cys Pro  Pro Asp Lys Val Met  Leu Glu Gly Ser Cys  Val Pro Glu
    1475                1480                1485

Glu Ala  Cys Thr Gln Cys Ile  Gly Glu Asp Gly Val  Gln His Gln
    1490                1495                1500

Phe Leu  Glu Ala Trp Val Pro  Asp His Gln Pro Cys  Gln Ile Cys
    1505                1510                1515

Thr Cys  Leu Ser Gly Arg Lys  Val Asn Cys Thr Thr  Gln Pro Cys
    1520                1525                1530

Pro Thr  Ala Lys Ala Pro Thr  Cys Gly Leu Cys Glu  Val Ala Arg
    1535                1540                1545

Leu Arg  Gln Asn Ala Asp Gln  Cys Cys Pro Glu Tyr  Glu Cys Val
    1550                1555                1560

Cys Asp  Pro Val Ser Cys Asp  Leu Pro Pro Val Pro  His Cys Glu
    1565                1570                1575

Arg Gly  Leu Gln Pro Thr Leu  Thr Asn Pro Gly Glu  Cys Arg Pro
    1580                1585                1590

Asn Phe  Thr Cys Ala Cys Arg  Lys Glu Glu Cys Lys  Arg Val Ser
    1595                1600                1605

Pro Pro  Ser Cys Pro Pro His  Arg Leu Pro Thr Leu  Arg Lys Thr
    1610                1615                1620

Gln Cys  Cys Asp Glu Tyr Glu  Cys Ala Cys Asn Cys  Val Asn Ser
    1625                1630                1635

Thr Val  Ser Cys Pro Leu Gly  Tyr Leu Ala Ser Thr  Ala Thr Asn
    1640                1645                1650

Asp Cys  Gly Cys Thr Thr Thr  Thr Cys Leu Pro Asp  Lys Val Cys
    1655                1660                1665

Val His  Arg Ser Thr Ile Tyr  Pro Val Gly Gln Phe  Trp Glu Glu
    1670                1675                1680

Gly Cys  Asp Val Cys Thr Cys  Thr Asp Met Glu Asp  Ala Val Met
    1685                1690                1695

Gly Leu  Arg Val Ala Gln Cys  Ser Gln Lys Pro Cys  Glu Asp Ser
    1700                1705                1710

Cys Arg  Ser Gly Phe Thr Tyr  Val Leu His Glu Gly  Glu Cys Cys
    1715                1720                1725

Gly Arg  Cys Leu Pro Ser Ala  Cys Glu Val Val Thr  Gly Ser Pro
    1730                1735                1740

Arg Gly  Asp Ser Gln Ser Ser  Trp Lys Ser Val Gly  Ser Gln Trp
    1745                1750                1755

Ala Ser  Pro Glu Asn Pro Cys  Leu Ile Asn Glu Cys  Val Arg Val
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    1760                1765                1770

Lys Glu  Glu Val Phe Ile Gln  Gln Arg Asn Val Ser  Cys Pro Gln
    1775                1780                1785

Leu Glu  Val Pro Val Cys Pro  Ser Gly Phe Gln Leu  Ser Cys Lys
    1790                1795                1800

Thr Ser  Ala Cys Cys Pro Ser  Cys Arg Cys Glu Arg  Met Glu Ala
    1805                1810                1815

Cys Met  Leu Asn Gly Thr Val  Ile Gly Pro Gly Lys  Thr Val Met
    1820                1825                1830

Ile Asp  Val Cys Thr Thr Cys  Arg Cys Met Val Gln  Val Gly Val
    1835                1840                1845

Ile Ser  Gly Phe Lys Leu Glu  Cys Arg Lys Thr Thr  Cys Asn Pro
    1850                1855                1860

Cys Pro  Leu Gly Tyr Lys Glu  Glu Asn Asn Thr Gly  Glu Cys Cys
    1865                1870                1875

Gly Arg  Cys Leu Pro Thr Ala  Cys Thr Ile Gln Leu  Arg Gly Gly
    1880                1885                1890

Gln Ile  Met Thr Leu Lys Arg  Asp Glu Thr Leu Gln  Asp Gly Cys
    1895                1900                1905

Asp Thr  His Phe Cys Lys Val  Asn Glu Arg Gly Glu  Tyr Phe Trp
    1910                1915                1920

Glu Lys  Arg Val Thr Gly Cys  Pro Pro Phe Asp Glu  His Lys Cys
    1925                1930                1935

Leu Ala  Glu Gly Gly Lys Ile  Met Lys Ile Pro Gly  Thr Cys Cys
    1940                1945                1950

Asp Thr  Cys Glu Glu Pro Glu  Cys Asn Asp Ile Thr  Ala Arg Leu
    1955                1960                1965

Gln Tyr  Val Lys Val Gly Ser  Cys Lys Ser Glu Val  Glu Val Asp
    1970                1975                1980

Ile His  Tyr Cys Gln Gly Lys  Cys Ala Ser Lys Ala  Met Tyr Ser
    1985                1990                1995

Ile Asp  Ile Asn Asp Val Gln  Asp Gln Cys Ser Cys  Cys Ser Pro
    2000                2005                2010

Thr Arg  Thr Glu Pro Met Gln  Val Ala Leu His Cys  Thr Asn Gly
    2015                2020                2025

Ser Val  Val Tyr His Glu Val  Leu Asn Ala Met Glu  Cys Lys Cys
    2030                2035                2040

Ser Pro  Arg Lys Cys Ser Lys
    2045                2050


<210> SEQ ID NO 33
<211> LENGTH: 102
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Modified D' domain of VWF
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (16)..(16)
<223> OTHER INFORMATION: Xaa is any amino acid
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (18)..(18)
<223> OTHER INFORMATION: Xaa is Leu or Pro
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (24)..(24)
<223> OTHER INFORMATION: Xaa is any amino acid
<220> FEATURE:
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<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (26)..(26)
<223> OTHER INFORMATION: Xaa is any amino acid
<220> FEATURE:
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<222> LOCATION: (30)..(30)
<223> OTHER INFORMATION: Xaa is any amino acid
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<223> OTHER INFORMATION: Xaa is any amino acid
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<223> OTHER INFORMATION: Xaa is any amino acid
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<223> OTHER INFORMATION: Xaa is any amino acid
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<223> OTHER INFORMATION: Xaa is any amino acid
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<223> OTHER INFORMATION: Xaa is any amino acid
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<222> LOCATION: (62)..(62)
<223> OTHER INFORMATION: Xaa is any amino acid
<220> FEATURE:
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<222> LOCATION: (72)..(72)
<223> OTHER INFORMATION: Xaa is any amino acid
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (75)..(75)
<223> OTHER INFORMATION: Xaa is any amino acid
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (90)..(90)
<223> OTHER INFORMATION: Xaa is any amino acid

<400> SEQUENCE: 33

Ser Leu Ser Cys Arg Pro Pro Met Val Lys Leu Val Cys Pro Ala Xaa
1 5 10 15

Asn Xaa Arg Ala Glu Gly Leu Xaa Cys Xaa Lys Thr Cys Xaa Xaa Tyr
20 25 30

Xaa Leu Xaa Cys Met Ser Xaa Gly Cys Val Ser Gly Cys Leu Cys Pro
35 40 45

Pro Gly Met Val Arg His Xaa Xaa Arg Cys Val Ala Leu Xaa Arg Cys
50 55 60

Pro Cys Phe His Gln Gly Lys Xaa Tyr Ala Xaa Gly Glu Thr Val Lys
65 70 75 80

Ile Gly Cys Asn Thr Cys Val Cys Arg Xaa Arg Lys Trp Asn Cys Thr
85 90 95

Asp His Val Cys Asp Ala
100

<210> SEQ ID NO 34
<211> LENGTH: 102
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: modified D' domain
<220> FEATURE:

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<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (16)..(16)
<223> OTHER INFORMATION: Xaa is any amino acid
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (18)..(18)
<223> OTHER INFORMATION: Xaa is any amino acid
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
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<223> OTHER INFORMATION: Xaa is any amino acid
<220> FEATURE:
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<222> LOCATION: (30)..(30)
<223> OTHER INFORMATION: Xaa is any amino acid
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<223> OTHER INFORMATION: Xaa is any amino acid
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<223> OTHER INFORMATION: Xaa is any amino acid
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<223> OTHER INFORMATION: Xaa is any amino acid
<220> FEATURE:
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<222> LOCATION: (72)..(72)
<223> OTHER INFORMATION: Xaa is any amino acid
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<221> NAME/KEY: MISC_FEATURE
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<223> OTHER INFORMATION: Xaa is any amino acid
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (90)..(90)
<223> OTHER INFORMATION: Xaa is any amino acid

<400> SEQUENCE: 34

Ser Leu Ser Cys Arg Pro Pro Met Val Lys Leu Val Cys Pro Ala Xaa
1 5 10 15

Asn Xaa Arg Ala Glu Gly Leu Glu Cys Xaa Lys Thr Cys Xaa Xaa Tyr
20 25 30

Asp Leu Glu Cys Met Ser Xaa Gly Cys Val Ser Gly Cys Leu Cys Pro
35 40 45

Pro Gly Met Val Arg His Xaa Xaa Arg Cys Val Ala Leu Glu Arg Cys
50 55 60

Pro Cys Phe His Gln Gly Lys Xaa Tyr Ala Xaa Gly Glu Thr Val Lys
65 70 75 80

Ile Gly Cys Asn Thr Cys Val Cys Arg Xaa Arg Lys Trp Asn Cys Thr
85 90 95

Asp His Val Cys Asp Ala
100

<210> SEQ ID NO 35
<211> LENGTH: 102
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: modified D' domain
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (16)..(16)
<223> OTHER INFORMATION: Xaa is Asp or Asn
<220> FEATURE:

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<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (18)..(18)
<223> OTHER INFORMATION: Xaa is Leu or Pro
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (26)..(26)
<223> OTHER INFORMATION: Xaa is Thr or Ala
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (30)..(30)
<223> OTHER INFORMATION: Xaa is Gln or Arg
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (31)..(31)
<223> OTHER INFORMATION: Xaa is Asn or Lys
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (39)..(39)
<223> OTHER INFORMATION: Xaa is Met or Arg or Lys
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (55)..(55)
<223> OTHER INFORMATION: Xaa Glu or Ala or Lys
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (56)..(56)
<223> OTHER INFORMATION: Xaa Asn or Lys
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (72)..(72)
<223> OTHER INFORMATION: Xaa Glu or Gln
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (75)..(75)
<223> OTHER INFORMATION: Xaa Pro or Lys
<220> FEATURE:
<221> NAME/KEY: MISC_FEATURE
<222> LOCATION: (90)..(90)
<223> OTHER INFORMATION: Xaa Asp or Asn

<400> SEQUENCE: 35

Ser Leu Ser Cys Arg Pro Pro Met Val Lys Leu Val Cys Pro Ala Xaa
1 5 10 15

Asn Xaa Arg Ala Glu Gly Leu Glu Cys Xaa Lys Thr Cys Xaa Xaa Tyr
20 25 30

Asp Leu Glu Cys Met Ser Xaa Gly Cys Val Ser Gly Cys Leu Cys Pro
35 40 45

Pro Gly Met Val Arg His Xaa Xaa Arg Cys Val Ala Leu Glu Arg Cys
50 55 60

Pro Cys Phe His Gln Gly Lys Xaa Tyr Ala Xaa Gly Glu Thr Val Lys
65 70 75 80

Ile Gly Cys Asn Thr Cys Val Cys Arg Xaa Arg Lys Trp Asn Cys Thr
85 90 95

Asp His Val Cys Asp Ala
100

<210> SEQ ID NO 36
<211> LENGTH: 2332
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 36

Ala Thr Arg Arg Tyr Tyr Leu Gly Ala Val Glu Leu Ser Trp Asp Tyr
1 5 10 15

Met Gln Ser Asp Leu Gly Glu Leu Pro Val Asp Ala Arg Phe Pro Pro
20 25 30

Arg Val Pro Lys Ser Phe Pro Phe Asn Thr Ser Val Val Tyr Lys Lys
35 40 45

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Thr Leu Phe Val Glu Phe Thr Asp His Leu Phe Asn Ile Ala Lys Pro
    50                  55                  60

Arg Pro Pro Trp Met Gly Leu Leu Gly Pro Thr Ile Gln Ala Glu Val
65                  70                  75                  80

Tyr Asp Thr Val Val Ile Thr Leu Lys Asn Met Ala Ser His Pro Val
                85                  90                  95

Ser Leu His Ala Val Gly Val Ser Tyr Trp Lys Ala Ser Glu Gly Ala
            100                 105                 110

Glu Tyr Asp Asp Gln Thr Ser Gln Arg Glu Lys Glu Asp Asp Lys Val
        115                 120                 125

Phe Pro Gly Gly Ser His Thr Tyr Val Trp Gln Val Leu Lys Glu Asn
    130                 135                 140

Gly Pro Met Ala Ser Asp Pro Leu Cys Leu Thr Tyr Ser Tyr Leu Ser
145                 150                 155                 160

His Val Asp Leu Val Lys Asp Leu Asn Ser Gly Leu Ile Gly Ala Leu
                165                 170                 175

Leu Val Cys Arg Glu Gly Ser Leu Ala Lys Glu Lys Thr Gln Thr Leu
            180                 185                 190

His Lys Phe Ile Leu Leu Phe Ala Val Phe Asp Glu Gly Lys Ser Trp
        195                 200                 205

His Ser Glu Thr Lys Asn Ser Leu Met Gln Asp Arg Asp Ala Ala Ser
    210                 215                 220

Ala Arg Ala Trp Pro Lys Met His Thr Val Asn Gly Tyr Val Asn Arg
225                 230                 235                 240

Ser Leu Pro Gly Leu Ile Gly Cys His Arg Lys Ser Val Tyr Trp His
                245                 250                 255

Val Ile Gly Met Gly Thr Thr Pro Glu Val His Ser Ile Phe Leu Glu
            260                 265                 270

Gly His Thr Phe Leu Val Arg Asn His Arg Gln Ala Ser Leu Glu Ile
        275                 280                 285

Ser Pro Ile Thr Phe Leu Thr Ala Gln Thr Leu Leu Met Asp Leu Gly
    290                 295                 300

Gln Phe Leu Leu Phe Cys His Ile Ser Ser His Gln His Asp Gly Met
305                 310                 315                 320

Glu Ala Tyr Val Lys Val Asp Ser Cys Pro Glu Glu Pro Gln Leu Arg
                325                 330                 335

Met Lys Asn Asn Glu Glu Ala Glu Asp Tyr Asp Asp Asp Leu Thr Asp
            340                 345                 350

Ser Glu Met Asp Val Val Arg Phe Asp Asp Asp Asn Ser Pro Ser Phe
        355                 360                 365

Ile Gln Ile Arg Ser Val Ala Lys Lys His Pro Lys Thr Trp Val His
    370                 375                 380

Tyr Ile Ala Ala Glu Glu Glu Asp Trp Asp Tyr Ala Pro Leu Val Leu
385                 390                 395                 400

Ala Pro Asp Asp Arg Ser Tyr Lys Ser Gln Tyr Leu Asn Asn Gly Pro
                405                 410                 415

Gln Arg Ile Gly Arg Lys Tyr Lys Lys Val Arg Phe Met Ala Tyr Thr
            420                 425                 430

Asp Glu Thr Phe Lys Thr Arg Glu Ala Ile Gln His Glu Ser Gly Ile
        435                 440                 445

Leu Gly Pro Leu Leu Tyr Gly Glu Val Gly Asp Thr Leu Leu Ile Ile
    450                 455                 460
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Phe Lys Asn Gln Ala Ser Arg Pro Tyr Asn Ile Tyr Pro His Gly Ile
465                 470                 475                 480

Thr Asp Val Arg Pro Leu Tyr Ser Arg Arg Leu Pro Lys Gly Val Lys
                485                 490                 495

His Leu Lys Asp Phe Pro Ile Leu Pro Gly Glu Ile Phe Lys Tyr Lys
            500                 505                 510

Trp Thr Val Thr Val Glu Asp Gly Pro Thr Lys Ser Asp Pro Arg Cys
        515                 520                 525

Leu Thr Arg Tyr Tyr Ser Ser Phe Val Asn Met Glu Arg Asp Leu Ala
    530                 535                 540

Ser Gly Leu Ile Gly Pro Leu Leu Ile Cys Tyr Lys Glu Ser Val Asp
545                 550                 555                 560

Gln Arg Gly Asn Gln Ile Met Ser Asp Lys Arg Asn Val Ile Leu Phe
                565                 570                 575

Ser Val Phe Asp Glu Asn Arg Ser Trp Tyr Leu Thr Glu Asn Ile Gln
            580                 585                 590

Arg Phe Leu Pro Asn Pro Ala Gly Val Gln Leu Glu Asp Pro Glu Phe
        595                 600                 605

Gln Ala Ser Asn Ile Met His Ser Ile Asn Gly Tyr Val Phe Asp Ser
    610                 615                 620

Leu Gln Leu Ser Val Cys Leu His Glu Val Ala Tyr Trp Tyr Ile Leu
625                 630                 635                 640

Ser Ile Gly Ala Gln Thr Asp Phe Leu Ser Val Phe Phe Ser Gly Tyr
                645                 650                 655

Thr Phe Lys His Lys Met Val Tyr Glu Asp Thr Leu Thr Leu Phe Pro
            660                 665                 670

Phe Ser Gly Glu Thr Val Phe Met Ser Met Glu Asn Pro Gly Leu Trp
        675                 680                 685

Ile Leu Gly Cys His Asn Ser Asp Phe Arg Asn Arg Gly Met Thr Ala
    690                 695                 700

Leu Leu Lys Val Ser Ser Cys Asp Lys Asn Thr Gly Asp Tyr Tyr Glu
705                 710                 715                 720

Asp Ser Tyr Glu Asp Ile Ser Ala Tyr Leu Leu Ser Lys Asn Asn Ala
                725                 730                 735

Ile Glu Pro Arg Ser Phe Ser Gln Asn Ser Arg His Arg Ser Thr Arg
            740                 745                 750

Gln Lys Gln Phe Asn Ala Thr Thr Ile Pro Glu Asn Asp Ile Glu Lys
        755                 760                 765

Thr Asp Pro Trp Phe Ala His Arg Thr Pro Met Pro Lys Ile Gln Asn
    770                 775                 780

Val Ser Ser Ser Asp Leu Leu Met Leu Leu Arg Gln Ser Pro Thr Pro
785                 790                 795                 800

His Gly Leu Ser Leu Ser Asp Leu Gln Glu Ala Lys Tyr Glu Thr Phe
                805                 810                 815

Ser Asp Asp Pro Ser Pro Gly Ala Ile Asp Ser Asn Asn Ser Leu Ser
            820                 825                 830

Glu Met Thr His Phe Arg Pro Gln Leu His His Ser Gly Asp Met Val
        835                 840                 845

Phe Thr Pro Glu Ser Gly Leu Gln Leu Arg Leu Asn Glu Lys Leu Gly
    850                 855                 860

Thr Thr Ala Ala Thr Glu Leu Lys Lys Leu Asp Phe Lys Val Ser Ser
865                 870                 875                 880

Thr Ser Asn Asn Leu Ile Ser Thr Ile Pro Ser Asp Asn Leu Ala Ala
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                885                 890                 895

Gly Thr Asp Asn Thr Ser Ser Leu Gly Pro Pro Ser Met Pro Val His
            900                 905                 910

Tyr Asp Ser Gln Leu Asp Thr Thr Leu Phe Gly Lys Lys Ser Ser Pro
        915                 920                 925

Leu Thr Glu Ser Gly Gly Pro Leu Ser Leu Ser Glu Glu Asn Asn Asp
    930                 935                 940

Ser Lys Leu Leu Glu Ser Gly Leu Met Asn Ser Gln Glu Ser Ser Trp
945                 950                 955                 960

Gly Lys Asn Val Ser Ser Thr Glu Ser Gly Arg Leu Phe Lys Gly Lys
                965                 970                 975

Arg Ala His Gly Pro Ala Leu Leu Thr Lys Asp Asn Ala Leu Phe Lys
            980                 985                 990

Val Ser Ile Ser Leu Leu Lys Thr  Asn Lys Thr Ser Asn  Asn Ser Ala
        995                 1000                1005

Thr Asn  Arg Lys Thr His Ile  Asp Gly Pro Ser Leu  Leu Ile Glu
    1010                1015                1020

Asn Ser  Pro Ser Val Trp Gln  Asn Ile Leu Glu Ser  Asp Thr Glu
    1025                1030                1035

Phe Lys  Lys Val Thr Pro Leu  Ile His Asp Arg Met  Leu Met Asp
    1040                1045                1050

Lys Asn  Ala Thr Ala Leu Arg  Leu Asn His Met Ser  Asn Lys Thr
    1055                1060                1065

Thr Ser  Ser Lys Asn Met Glu  Met Val Gln Gln Lys  Lys Glu Gly
    1070                1075                1080

Pro Ile  Pro Pro Asp Ala Gln  Asn Pro Asp Met Ser  Phe Phe Lys
    1085                1090                1095

Met Leu  Phe Leu Pro Glu Ser  Ala Arg Trp Ile Gln  Arg Thr His
    1100                1105                1110

Gly Lys  Asn Ser Leu Asn Ser  Gly Gln Gly Pro Ser  Pro Lys Gln
    1115                1120                1125

Leu Val  Ser Leu Gly Pro Glu  Lys Ser Val Glu Gly  Gln Asn Phe
    1130                1135                1140

Leu Ser  Glu Lys Asn Lys Val  Val Val Gly Lys Gly  Glu Phe Thr
    1145                1150                1155

Lys Asp  Val Gly Leu Lys Glu  Met Val Phe Pro Ser  Ser Arg Asn
    1160                1165                1170

Leu Phe  Leu Thr Asn Leu Asp  Asn Leu His Glu Asn  Asn Thr His
    1175                1180                1185

Asn Gln  Glu Lys Lys Ile Gln  Glu Glu Ile Glu Lys  Lys Glu Thr
    1190                1195                1200

Leu Ile  Gln Glu Asn Val Val  Leu Pro Gln Ile His  Thr Val Thr
    1205                1210                1215

Gly Thr  Lys Asn Phe Met Lys  Asn Leu Phe Leu Leu  Ser Thr Arg
    1220                1225                1230

Gln Asn  Val Glu Gly Ser Tyr  Asp Gly Ala Tyr Ala  Pro Val Leu
    1235                1240                1245

Gln Asp  Phe Arg Ser Leu Asn  Asp Ser Thr Asn Arg  Thr Lys Lys
    1250                1255                1260

His Thr  Ala His Phe Ser Lys  Lys Gly Glu Glu Glu  Asn Leu Glu
    1265                1270                1275

Gly Leu  Gly Asn Gln Thr Lys  Gln Ile Val Glu Lys  Tyr Ala Cys
    1280                1285                1290
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Thr Thr  Arg Ile Ser Pro Asn  Thr Ser Gln Gln Asn  Phe Val Thr
    1295                 1300                 1305

Gln Arg  Ser Lys Arg Ala Leu  Lys Gln Phe Arg Leu  Pro Leu Glu
    1310                 1315                 1320

Glu Thr  Glu Leu Glu Lys Arg  Ile Ile Val Asp Asp  Thr Ser Thr
    1325                 1330                 1335

Gln Trp  Ser Lys Asn Met Lys  His Leu Thr Pro Ser  Thr Leu Thr
    1340                 1345                 1350

Gln Ile  Asp Tyr Asn Glu Lys  Glu Lys Gly Ala Ile  Thr Gln Ser
    1355                 1360                 1365

Pro Leu  Ser Asp Cys Leu Thr  Arg Ser His Ser Ile  Pro Gln Ala
    1370                 1375                 1380

Asn Arg  Ser Pro Leu Pro Ile  Ala Lys Val Ser Ser  Phe Pro Ser
    1385                 1390                 1395

Ile Arg  Pro Ile Tyr Leu Thr  Arg Val Leu Phe Gln  Asp Asn Ser
    1400                 1405                 1410

Ser His  Leu Pro Ala Ala Ser  Tyr Arg Lys Lys Asp  Ser Gly Val
    1415                 1420                 1425

Gln Glu  Ser Ser His Phe Leu  Gln Gly Ala Lys Lys  Asn Asn Leu
    1430                 1435                 1440

Ser Leu  Ala Ile Leu Thr Leu  Glu Met Thr Gly Asp  Gln Arg Glu
    1445                 1450                 1455

Val Gly  Ser Leu Gly Thr Ser  Ala Thr Asn Ser Val  Thr Tyr Lys
    1460                 1465                 1470

Lys Val  Glu Asn Thr Val Leu  Pro Lys Pro Asp Leu  Pro Lys Thr
    1475                 1480                 1485

Ser Gly  Lys Val Glu Leu Leu  Pro Lys Val His Ile  Tyr Gln Lys
    1490                 1495                 1500

Asp Leu  Phe Pro Thr Glu Thr  Ser Asn Gly Ser Pro  Gly His Leu
    1505                 1510                 1515

Asp Leu  Val Glu Gly Ser Leu  Leu Gln Gly Thr Glu  Gly Ala Ile
    1520                 1525                 1530

Lys Trp  Asn Glu Ala Asn Arg  Pro Gly Lys Val Pro  Phe Leu Arg
    1535                 1540                 1545

Val Ala  Thr Glu Ser Ser Ala  Lys Thr Pro Ser Lys  Leu Leu Asp
    1550                 1555                 1560

Pro Leu  Ala Trp Asp Asn His  Tyr Gly Thr Gln Ile  Pro Lys Glu
    1565                 1570                 1575

Glu Trp  Lys Ser Gln Glu Lys  Ser Pro Glu Lys Thr  Ala Phe Lys
    1580                 1585                 1590

Lys Lys  Asp Thr Ile Leu Ser  Leu Asn Ala Cys Glu  Ser Asn His
    1595                 1600                 1605

Ala Ile  Ala Ala Ile Asn Glu  Gly Gln Asn Lys Pro  Glu Ile Glu
    1610                 1615                 1620

Val Thr  Trp Ala Lys Gln Gly  Arg Thr Glu Arg Leu  Cys Ser Gln
    1625                 1630                 1635

Asn Pro  Pro Val Leu Lys Arg  His Gln Arg Glu Ile  Thr Arg Thr
    1640                 1645                 1650

Thr Leu  Gln Ser Asp Gln Glu  Glu Ile Asp Tyr Asp  Asp Thr Ile
    1655                 1660                 1665

Ser Val  Glu Met Lys Lys Glu  Asp Phe Asp Ile Tyr  Asp Glu Asp
    1670                 1675                 1680
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Glu Asn  Gln Ser Pro Arg Ser  Phe Gln Lys Lys Thr  Arg His Tyr
    1685                 1690                 1695

Phe Ile  Ala Ala Val Glu Arg  Leu Trp Asp Tyr Gly  Met Ser Ser
    1700                 1705                 1710

Ser Pro  His Val Leu Arg Asn  Arg Ala Gln Ser Gly  Ser Val Pro
    1715                 1720                 1725

Gln Phe  Lys Lys Val Val Phe  Gln Glu Phe Thr Asp  Gly Ser Phe
    1730                 1735                 1740

Thr Gln  Pro Leu Tyr Arg Gly  Glu Leu Asn Glu His  Leu Gly Leu
    1745                 1750                 1755

Leu Gly  Pro Tyr Ile Arg Ala  Glu Val Glu Asp Asn  Ile Met Val
    1760                 1765                 1770

Thr Phe  Arg Asn Gln Ala Ser  Arg Pro Tyr Ser Phe  Tyr Ser Ser
    1775                 1780                 1785

Leu Ile  Ser Tyr Glu Glu Asp  Gln Arg Gln Gly Ala  Glu Pro Arg
    1790                 1795                 1800

Lys Asn  Phe Val Lys Pro Asn  Glu Thr Lys Thr Tyr  Phe Trp Lys
    1805                 1810                 1815

Val Gln  His His Met Ala Pro  Thr Lys Asp Glu Phe  Asp Cys Lys
    1820                 1825                 1830

Ala Trp  Ala Tyr Phe Ser Asp  Val Asp Leu Glu Lys  Asp Val His
    1835                 1840                 1845

Ser Gly  Leu Ile Gly Pro Leu  Leu Val Cys His Thr  Asn Thr Leu
    1850                 1855                 1860

Asn Pro  Ala His Gly Arg Gln  Val Thr Val Gln Glu  Phe Ala Leu
    1865                 1870                 1875

Phe Phe  Thr Ile Phe Asp Glu  Thr Lys Ser Trp Tyr  Phe Thr Glu
    1880                 1885                 1890

Asn Met  Glu Arg Asn Cys Arg  Ala Pro Cys Asn Ile  Gln Met Glu
    1895                 1900                 1905

Asp Pro  Thr Phe Lys Glu Asn  Tyr Arg Phe His Ala  Ile Asn Gly
    1910                 1915                 1920

Tyr Ile  Met Asp Thr Leu Pro  Gly Leu Val Met Ala  Gln Asp Gln
    1925                 1930                 1935

Arg Ile  Arg Trp Tyr Leu Leu  Ser Met Gly Ser Asn  Glu Asn Ile
    1940                 1945                 1950

His Ser  Ile His Phe Ser Gly  His Val Phe Thr Val  Arg Lys Lys
    1955                 1960                 1965

Glu Glu  Tyr Lys Met Ala Leu  Tyr Asn Leu Tyr Pro  Gly Val Phe
    1970                 1975                 1980

Glu Thr  Val Glu Met Leu Pro  Ser Lys Ala Gly Ile  Trp Arg Val
    1985                 1990                 1995

Glu Cys  Leu Ile Gly Glu His  Leu His Ala Gly Met  Ser Thr Leu
    2000                 2005                 2010

Phe Leu  Val Tyr Ser Asn Lys  Cys Gln Thr Pro Leu  Gly Met Ala
    2015                 2020                 2025

Ser Gly  His Ile Arg Asp Phe  Gln Ile Thr Ala Ser  Gly Gln Tyr
    2030                 2035                 2040

Gly Gln  Trp Ala Pro Lys Leu  Ala Arg Leu His Tyr  Ser Gly Ser
    2045                 2050                 2055

Ile Asn  Ala Trp Ser Thr Lys  Glu Pro Phe Ser Trp  Ile Lys Val
    2060                 2065                 2070

Asp Leu  Leu Ala Pro Met Ile  Ile His Gly Ile Lys  Thr Gln Gly
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    2075                2080                2085

Ala Arg  Gln Lys Phe Ser Ser  Leu Tyr Ile Ser Gln  Phe Ile Ile
    2090                2095                2100

Met Tyr  Ser Leu Asp Gly Lys  Lys Trp Gln Thr Tyr  Arg Gly Asn
    2105                2110                2115

Ser Thr  Gly Thr Leu Met Val  Phe Phe Gly Asn Val  Asp Ser Ser
    2120                2125                2130

Gly Ile  Lys His Asn Ile Phe  Asn Pro Pro Ile Ile  Ala Arg Tyr
    2135                2140                2145

Ile Arg  Leu His Pro Thr His  Tyr Ser Ile Arg Ser  Thr Leu Arg
    2150                2155                2160

Met Glu  Leu Met Gly Cys Asp  Leu Asn Ser Cys Ser  Met Pro Leu
    2165                2170                2175

Gly Met  Glu Ser Lys Ala Ile  Ser Asp Ala Gln Ile  Thr Ala Ser
    2180                2185                2190

Ser Tyr  Phe Thr Asn Met Phe  Ala Thr Trp Ser Pro  Ser Lys Ala
    2195                2200                2205

Arg Leu  His Leu Gln Gly Arg  Ser Asn Ala Trp Arg  Pro Gln Val
    2210                2215                2220

Asn Asn  Pro Lys Glu Trp Leu  Gln Val Asp Phe Gln  Lys Thr Met
    2225                2230                2235

Lys Val  Thr Gly Val Thr Thr  Gln Gly Val Lys Ser  Leu Leu Thr
    2240                2245                2250

Ser Met  Tyr Val Lys Glu Phe  Leu Ile Ser Ser Ser  Gln Asp Gly
    2255                2260                2265

His Gln  Trp Thr Leu Phe Phe  Gln Asn Gly Lys Val  Lys Val Phe
    2270                2275                2280

Gln Gly  Asn Gln Asp Ser Phe  Thr Pro Val Val Asn  Ser Leu Asp
    2285                2290                2295

Pro Pro  Leu Leu Thr Arg Tyr  Leu Arg Ile His Pro  Gln Ser Trp
    2300                2305                2310

Val His  Gln Ile Ala Leu Arg  Met Glu Val Leu Gly  Cys Glu Ala
    2315                2320                2325

Gln Asp  Leu Tyr
    2330


<210> SEQ ID NO 37
<211> LENGTH: 1444
<212> TYPE: PRT
<213> ORGANISM: artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: mature single chain Factor VIII

<400> SEQUENCE: 37

Ala Thr Arg Arg Tyr Tyr Leu Gly Ala Val Glu Leu Ser Trp Asp Tyr
1               5                  10                  15

Met Gln Ser Asp Leu Gly Glu Leu Pro Val Asp Ala Arg Phe Pro Pro
            20                  25                  30

Arg Val Pro Lys Ser Phe Pro Phe Asn Thr Ser Val Val Tyr Lys Lys
        35                  40                  45

Thr Leu Phe Val Glu Phe Thr Asp His Leu Phe Asn Ile Ala Lys Pro
    50                  55                  60

Arg Pro Pro Trp Met Gly Leu Leu Gly Pro Thr Ile Gln Ala Glu Val
65                  70                  75                  80

Tyr Asp Thr Val Val Ile Thr Leu Lys Asn Met Ala Ser His Pro Val
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               85                  90                  95

Ser Leu His Ala Val Gly Val Ser Tyr Trp Lys Ala Ser Glu Gly Ala
            100                 105                 110

Glu Tyr Asp Asp Gln Thr Ser Gln Arg Glu Lys Glu Asp Asp Lys Val
        115                 120                 125

Phe Pro Gly Gly Ser His Thr Tyr Val Trp Gln Val Leu Lys Glu Asn
    130                 135                 140

Gly Pro Met Ala Ser Asp Pro Leu Cys Leu Thr Tyr Ser Tyr Leu Ser
145                 150                 155                 160

His Val Asp Leu Val Lys Asp Leu Asn Ser Gly Leu Ile Gly Ala Leu
               165                 170                 175

Leu Val Cys Arg Glu Gly Ser Leu Ala Lys Glu Lys Thr Gln Thr Leu
            180                 185                 190

His Lys Phe Ile Leu Leu Phe Ala Val Phe Asp Glu Gly Lys Ser Trp
        195                 200                 205

His Ser Glu Thr Lys Asn Ser Leu Met Gln Asp Arg Asp Ala Ala Ser
    210                 215                 220

Ala Arg Ala Trp Pro Lys Met His Thr Val Asn Gly Tyr Val Asn Arg
225                 230                 235                 240

Ser Leu Pro Gly Leu Ile Gly Cys His Arg Lys Ser Val Tyr Trp His
               245                 250                 255

Val Ile Gly Met Gly Thr Thr Pro Glu Val His Ser Ile Phe Leu Glu
            260                 265                 270

Gly His Thr Phe Leu Val Arg Asn His Arg Gln Ala Ser Leu Glu Ile
        275                 280                 285

Ser Pro Ile Thr Phe Leu Thr Ala Gln Thr Leu Leu Met Asp Leu Gly
    290                 295                 300

Gln Phe Leu Leu Phe Cys His Ile Ser Ser His Gln His Asp Gly Met
305                 310                 315                 320

Glu Ala Tyr Val Lys Val Asp Ser Cys Pro Glu Glu Pro Gln Leu Arg
               325                 330                 335

Met Lys Asn Asn Glu Glu Ala Glu Asp Tyr Asp Asp Asp Leu Thr Asp
            340                 345                 350

Ser Glu Met Asp Val Val Arg Phe Asp Asp Asp Asn Ser Pro Ser Phe
        355                 360                 365

Ile Gln Ile Arg Ser Val Ala Lys Lys His Pro Lys Thr Trp Val His
    370                 375                 380

Tyr Ile Ala Ala Glu Glu Glu Asp Trp Asp Tyr Ala Pro Leu Val Leu
385                 390                 395                 400

Ala Pro Asp Asp Arg Ser Tyr Lys Ser Gln Tyr Leu Asn Asn Gly Pro
               405                 410                 415

Gln Arg Ile Gly Arg Lys Tyr Lys Lys Val Arg Phe Met Ala Tyr Thr
            420                 425                 430

Asp Glu Thr Phe Lys Thr Arg Glu Ala Ile Gln His Glu Ser Gly Ile
        435                 440                 445

Leu Gly Pro Leu Leu Tyr Gly Glu Val Gly Asp Thr Leu Leu Ile Ile
    450                 455                 460

Phe Lys Asn Gln Ala Ser Arg Pro Tyr Asn Ile Tyr Pro His Gly Ile
465                 470                 475                 480

Thr Asp Val Arg Pro Leu Tyr Ser Arg Arg Leu Pro Lys Gly Val Lys
               485                 490                 495

His Leu Lys Asp Phe Pro Ile Leu Pro Gly Glu Ile Phe Lys Tyr Lys
            500                 505                 510
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Trp Thr Val Thr Val Glu Asp Gly Pro Thr Lys Ser Asp Pro Arg Cys
        515                 520                 525

Leu Thr Arg Tyr Tyr Ser Ser Phe Val Asn Met Glu Arg Asp Leu Ala
    530                 535                 540

Ser Gly Leu Ile Gly Pro Leu Leu Ile Cys Tyr Lys Glu Ser Val Asp
545                 550                 555                 560

Gln Arg Gly Asn Gln Ile Met Ser Asp Lys Arg Asn Val Ile Leu Phe
                565                 570                 575

Ser Val Phe Asp Glu Asn Arg Ser Trp Tyr Leu Thr Glu Asn Ile Gln
            580                 585                 590

Arg Phe Leu Pro Asn Pro Ala Gly Val Gln Leu Glu Asp Pro Glu Phe
        595                 600                 605

Gln Ala Ser Asn Ile Met His Ser Ile Asn Gly Tyr Val Phe Asp Ser
    610                 615                 620

Leu Gln Leu Ser Val Cys Leu His Glu Val Ala Tyr Trp Tyr Ile Leu
625                 630                 635                 640

Ser Ile Gly Ala Gln Thr Asp Phe Leu Ser Val Phe Phe Ser Gly Tyr
                645                 650                 655

Thr Phe Lys His Lys Met Val Tyr Glu Asp Thr Leu Thr Leu Phe Pro
            660                 665                 670

Phe Ser Gly Glu Thr Val Phe Met Ser Met Glu Asn Pro Gly Leu Trp
        675                 680                 685

Ile Leu Gly Cys His Asn Ser Asp Phe Arg Asn Arg Gly Met Thr Ala
    690                 695                 700

Leu Leu Lys Val Ser Ser Cys Asp Lys Asn Thr Gly Asp Tyr Tyr Glu
705                 710                 715                 720

Asp Ser Tyr Glu Asp Ile Ser Ala Tyr Leu Leu Ser Lys Asn Asn Ala
                725                 730                 735

Ile Glu Pro Arg Ser Phe Ser Gln Asn Ser Arg His Arg Ser Thr Arg
            740                 745                 750

Gln Lys Gln Phe Asn Ala Thr Thr Ile Pro Glu Asn Thr Thr Leu Gln
        755                 760                 765

Ser Asp Gln Glu Glu Ile Asp Tyr Asp Asp Thr Ile Ser Val Glu Met
    770                 775                 780

Lys Lys Glu Asp Phe Asp Ile Tyr Asp Glu Asp Glu Asn Gln Ser Pro
785                 790                 795                 800

Arg Ser Phe Gln Lys Lys Thr Arg His Tyr Phe Ile Ala Ala Val Glu
                805                 810                 815

Arg Leu Trp Asp Tyr Gly Met Ser Ser Ser Pro His Val Leu Arg Asn
            820                 825                 830

Arg Ala Gln Ser Gly Ser Val Pro Gln Phe Lys Lys Val Val Phe Gln
        835                 840                 845

Glu Phe Thr Asp Gly Ser Phe Thr Gln Pro Leu Tyr Arg Gly Glu Leu
    850                 855                 860

Asn Glu His Leu Gly Leu Leu Gly Pro Tyr Ile Arg Ala Glu Val Glu
865                 870                 875                 880

Asp Asn Ile Met Val Thr Phe Arg Asn Gln Ala Ser Arg Pro Tyr Ser
                885                 890                 895

Phe Tyr Ser Ser Leu Ile Ser Tyr Glu Glu Asp Gln Arg Gln Gly Ala
            900                 905                 910

Glu Pro Arg Lys Asn Phe Val Lys Pro Asn Glu Thr Lys Thr Tyr Phe
        915                 920                 925
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Trp Lys Val Gln His His Met Ala Pro Thr Lys Asp Glu Phe Asp Cys
    930                 935                 940

Lys Ala Trp Ala Tyr Phe Ser Asp Val Asp Leu Glu Lys Asp Val His
945                 950                 955                 960

Ser Gly Leu Ile Gly Pro Leu Leu Val Cys His Thr Asn Thr Leu Asn
                965                 970                 975

Pro Ala His Gly Arg Gln Val Thr Val Gln Glu Phe Ala Leu Phe Phe
            980                 985                 990

Thr Ile Phe Asp Glu Thr Lys Ser  Trp Tyr Phe Thr Glu  Asn Met Glu
        995                 1000                1005

Arg Asn  Cys Arg Ala Pro Cys  Asn Ile Gln Met Glu  Asp Pro Thr
    1010                1015                1020

Phe Lys  Glu Asn Tyr Arg Phe  His Ala Ile Asn Gly  Tyr Ile Met
    1025                1030                1035

Asp Thr  Leu Pro Gly Leu Val  Met Ala Gln Asp Gln  Arg Ile Arg
    1040                1045                1050

Trp Tyr  Leu Leu Ser Met Gly  Ser Asn Glu Asn Ile  His Ser Ile
    1055                1060                1065

His Phe  Ser Gly His Val Phe  Thr Val Arg Lys Lys  Glu Glu Tyr
    1070                1075                1080

Lys Met  Ala Leu Tyr Asn Leu  Tyr Pro Gly Val Phe  Glu Thr Val
    1085                1090                1095

Glu Met  Leu Pro Ser Lys Ala  Gly Ile Trp Arg Val  Glu Cys Leu
    1100                1105                1110

Ile Gly  Glu His Leu His Ala  Gly Met Ser Thr Leu  Phe Leu Val
    1115                1120                1125

Tyr Ser  Asn Lys Cys Gln Thr  Pro Leu Gly Met Ala  Ser Gly His
    1130                1135                1140

Ile Arg  Asp Phe Gln Ile Thr  Ala Ser Gly Gln Tyr  Gly Gln Trp
    1145                1150                1155

Ala Pro  Lys Leu Ala Arg Leu  His Tyr Ser Gly Ser  Ile Asn Ala
    1160                1165                1170

Trp Ser  Thr Lys Glu Pro Phe  Ser Trp Ile Lys Val  Asp Leu Leu
    1175                1180                1185

Ala Pro  Met Ile Ile His Gly  Ile Lys Thr Gln Gly  Ala Arg Gln
    1190                1195                1200

Lys Phe  Ser Ser Leu Tyr Ile  Ser Gln Phe Ile Ile  Met Tyr Ser
    1205                1210                1215

Leu Asp  Gly Lys Lys Trp Gln  Thr Tyr Arg Gly Asn  Ser Thr Gly
    1220                1225                1230

Thr Leu  Met Val Phe Phe Gly  Asn Val Asp Ser Ser  Gly Ile Lys
    1235                1240                1245

His Asn  Ile Phe Asn Pro Pro  Ile Ile Ala Arg Tyr  Ile Arg Leu
    1250                1255                1260

His Pro  Thr His Tyr Ser Ile  Arg Ser Thr Leu Arg  Met Glu Leu
    1265                1270                1275

Met Gly  Cys Asp Leu Asn Ser  Cys Ser Met Pro Leu  Gly Met Glu
    1280                1285                1290

Ser Lys  Ala Ile Ser Asp Ala  Gln Ile Thr Ala Ser  Ser Tyr Phe
    1295                1300                1305

Thr Asn  Met Phe Ala Thr Trp  Ser Pro Ser Lys Ala  Arg Leu His
    1310                1315                1320

Leu Gln  Gly Arg Ser Asn Ala  Trp Arg Pro Gln Val  Asn Asn Pro
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    1325                1330                1335

Lys Glu  Trp Leu Gln Val Asp  Phe Gln Lys Thr Met  Lys Val Thr
    1340                1345                1350

Gly Val  Thr Thr Gln Gly Val  Lys Ser Leu Leu Thr  Ser Met Tyr
    1355                1360                1365

Val Lys  Glu Phe Leu Ile Ser  Ser Ser Gln Asp Gly  His Gln Trp
    1370                1375                1380

Thr Leu  Phe Phe Gln Asn Gly  Lys Val Lys Val Phe  Gln Gly Asn
    1385                1390                1395

Gln Asp  Ser Phe Thr Pro Val  Val Asn Ser Leu Asp  Pro Pro Leu
    1400                1405                1410

Leu Thr  Arg Tyr Leu Arg Ile  His Pro Gln Ser Trp  Val His Gln
    1415                1420                1425

Ile Ala  Leu Arg Met Glu Val  Leu Gly Cys Glu Ala  Gln Asp Leu
    1430                1435                1440

Tyr


<210> SEQ ID NO 38
<211> LENGTH: 585
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 38

Asp Ala His Lys Ser Glu Val Ala His Arg Phe Lys Asp Leu Gly Glu
1               5                   10                  15

Glu Asn Phe Lys Ala Leu Val Leu Ile Ala Phe Ala Gln Tyr Leu Gln
            20                  25                  30

Gln Cys Pro Phe Glu Asp His Val Lys Leu Val Asn Glu Val Thr Glu
        35                  40                  45

Phe Ala Lys Thr Cys Val Ala Asp Glu Ser Ala Glu Asn Cys Asp Lys
    50                  55                  60

Ser Leu His Thr Leu Phe Gly Asp Lys Leu Cys Thr Val Ala Thr Leu
65                  70                  75                  80

Arg Glu Thr Tyr Gly Glu Met Ala Asp Cys Cys Ala Lys Gln Glu Pro
                85                  90                  95

Glu Arg Asn Glu Cys Phe Leu Gln His Lys Asp Asp Asn Pro Asn Leu
            100                 105                 110

Pro Arg Leu Val Arg Pro Glu Val Asp Val Met Cys Thr Ala Phe His
        115                 120                 125

Asp Asn Glu Glu Thr Phe Leu Lys Lys Tyr Leu Tyr Glu Ile Ala Arg
    130                 135                 140

Arg His Pro Tyr Phe Tyr Ala Pro Glu Leu Leu Phe Phe Ala Lys Arg
145                 150                 155                 160

Tyr Lys Ala Ala Phe Thr Glu Cys Cys Gln Ala Ala Asp Lys Ala Ala
                165                 170                 175

Cys Leu Leu Pro Lys Leu Asp Glu Leu Arg Asp Glu Gly Lys Ala Ser
            180                 185                 190

Ser Ala Lys Gln Arg Leu Lys Cys Ala Ser Leu Gln Lys Phe Gly Glu
        195                 200                 205

Arg Ala Phe Lys Ala Trp Ala Val Ala Arg Leu Ser Gln Arg Phe Pro
    210                 215                 220

Lys Ala Glu Phe Ala Glu Val Ser Lys Leu Val Thr Asp Leu Thr Lys
225                 230                 235                 240

Val His Thr Glu Cys Cys His Gly Asp Leu Leu Glu Cys Ala Asp Asp
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                245                 250                 255

Arg Ala Asp Leu Ala Lys Tyr Ile Cys Glu Asn Gln Asp Ser Ile Ser
            260                 265                 270

Ser Lys Leu Lys Glu Cys Cys Glu Lys Pro Leu Leu Glu Lys Ser His
        275                 280                 285

Cys Ile Ala Glu Val Glu Asn Asp Glu Met Pro Ala Asp Leu Pro Ser
    290                 295                 300

Leu Ala Ala Asp Phe Val Glu Ser Lys Asp Val Cys Lys Asn Tyr Ala
305                 310                 315                 320

Glu Ala Lys Asp Val Phe Leu Gly Met Phe Leu Tyr Glu Tyr Ala Arg
                325                 330                 335

Arg His Pro Asp Tyr Ser Val Val Leu Leu Leu Arg Leu Ala Lys Thr
            340                 345                 350

Tyr Glu Thr Thr Leu Glu Lys Cys Cys Ala Ala Ala Asp Pro His Glu
        355                 360                 365

Cys Tyr Ala Lys Val Phe Asp Glu Phe Lys Pro Leu Val Glu Glu Pro
    370                 375                 380

Gln Asn Leu Ile Lys Gln Asn Cys Glu Leu Phe Glu Gln Leu Gly Glu
385                 390                 395                 400

Tyr Lys Phe Gln Asn Ala Leu Leu Val Arg Tyr Thr Lys Lys Val Pro
                405                 410                 415

Gln Val Ser Thr Pro Thr Leu Val Glu Val Ser Arg Asn Leu Gly Lys
            420                 425                 430

Val Gly Ser Lys Cys Cys Lys His Pro Glu Ala Lys Arg Met Pro Cys
        435                 440                 445

Ala Glu Asp Tyr Leu Ser Val Val Leu Asn Gln Leu Cys Val Leu His
    450                 455                 460

Glu Lys Thr Pro Val Ser Asp Arg Val Thr Lys Cys Cys Thr Glu Ser
465                 470                 475                 480

Leu Val Asn Arg Arg Pro Cys Phe Ser Ala Leu Glu Val Asp Glu Thr
                485                 490                 495

Tyr Val Pro Lys Glu Phe Asn Ala Glu Thr Phe Thr Phe His Ala Asp
            500                 505                 510

Ile Cys Thr Leu Ser Glu Lys Glu Arg Gln Ile Lys Lys Gln Thr Ala
        515                 520                 525

Leu Val Glu Leu Val Lys His Lys Pro Lys Ala Thr Lys Glu Gln Leu
    530                 535                 540

Lys Ala Val Met Asp Asp Phe Ala Ala Phe Val Glu Lys Cys Cys Lys
545                 550                 555                 560

Ala Asp Asp Lys Glu Thr Cys Phe Ala Glu Glu Gly Lys Lys Leu Val
                565                 570                 575

Ala Ala Ser Gln Ala Ala Leu Gly Leu
            580                 585
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The invention claimed is:

1. A polypeptide comprising a modified von Willebrand Factor (VWF),

wherein the amino acid sequence of the modified VWF is identical to a human VWF sequence except for a modified D' domain, wherein the modified D' domain has at least one mutation relative to the amino acid sequence of positions 764 to 865 of SEQ ID NO:2,

wherein the at least one mutation within the D' domain comprises at least one amino acid substitution at a position selected from the group consisting of 779, 781, 793, 794, 796, 798, 802, 818, 819, 825, 835, 838 and 853 of the VWF amino acid sequence as shown in SEQ ID NO:2,

and wherein the binding affinity of the polypeptide comprising the modified VWF to Factor VIII (FVIII) is higher than that of a reference human VWF polypeptide, wherein the amino acid sequence of the reference polypeptide comprises positions 764 to 865 of SEQ ID NO:2.

2. The polypeptide of claim **1**, wherein the amino acid substitution within the D' domain is selected from the group

consisting of Asp779Asn, Leu781Pro, Gln793Arg, Asn794Lys, Met802Arg, Met802Lys, Glu818Ala, Glu818Lys, Asn819Lys, Glu835Gln, Pro838Lys, and Asp853Asn, wherein the amino acid numbering refers to SEQ ID NO:2.

3. The polypeptide of claim **1**, further comprising a half-life enhancing protein (HLEP).

4. The polypeptide of claim **3**, wherein said HLEP is an albumin.

5. The polypeptide of claim **4**, wherein the N-terminus of the albumin is fused to the C-terminus of the modified VWF amino acid sequence.

6. A pharmaceutical composition comprising the polypeptide of claim **1**.

7. A complex comprising a Factor VIII molecule and the polypeptide of claim **1**.

8. The complex of claim **7** wherein the Factor VIII molecule comprises the polypeptide of SEQ ID NO:37, and wherein the dissociation constant K_D of the complex is less than 90% of the dissociation constant K_D of a complex of the reference polypeptide and the Factor VIII molecule of SEQ ID NO:37, wherein the amino acid sequence of the reference polypeptide comprises positions 764 to 865 of SEQ ID NO:2.

9. A method of treating a bleeding disorder, comprising administering to a patient in need thereof a pharmaceutically effective amount of the polypeptide of claim **1**.

10. The method of claim **9**, wherein the bleeding disorder is von Willebrand's disease (VWD) or hemophilia A.

11. A method of increasing the Factor VIII binding affinity of VWF, comprising modifying a human VWF in a D' domain, wherein the modified D' domain has at least one mutation relative to the amino acid sequence of positions 764 to 865 of SEQ ID NO:2,

wherein the at least one mutation within the D' domain comprises at least one amino acid substitution at a position selected from the group consisting of 779, 781, 793, 794, 796, 798, 802, 818, 819, 825, 835, 838 and 853 of the VWF amino acid sequence as shown in SEQ ID NO:2,

and wherein the binding affinity of the polypeptide comprising the modified VWF to Factor VIII (FVIII) is higher than that of a reference human VWF polypeptide, wherein the amino acid sequence of the reference polypeptide comprises positions 764 to 865 of SEQ ID NO:2.

12. The method of claim **11**, wherein at least one acidic residue of the VWF D' domain is replaced with a neutral or basic amino acid, or wherein at least one neutral residue of the VWF D' domain is replaced with a basic amino acid.

13. A method for increasing the half-life of Factor VIII, comprising mixing the Factor VIII with the modified VWF polypeptide of claim **1**.

14. The method of claim **13**, wherein said modified VWF polypeptide further comprises a half-life enhancing protein (HLEP).

15. A method of preparing a complex comprising FVIII and a modified VWF, comprising mixing a Factor VIII polypeptide with the polypeptide of claim **1**.

16. A polynucleotide encoding the polypeptide of claim **1**.

17. A plasmid or vector comprising the polynucleotide of claim **16**.

18. The plasmid or vector of claim **17**, said plasmid or vector being an expression vector.

19. A host cell comprising the polynucleotide of claim **16**.

20. A method of producing a modified VWF, comprising:

(a) culturing the host cell of claim **19** under conditions such that the modified VWF is expressed; and

(b) optionally recovering the modified VWF from the host cells or from the culture medium.

* * * * *